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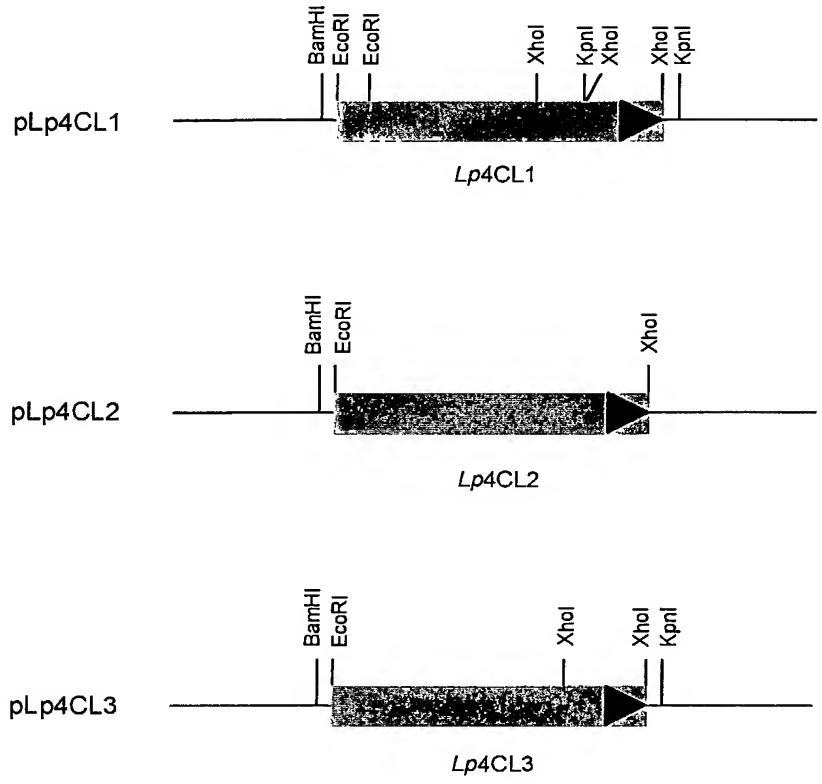
(54) Title: MODIFICATION OF LIGNIN BIOSYNTHESIS



(57) Abstract: The present invention relates to the modification of lignin biosynthesis in plants, using the nucleotide sequences encoding the enzymes 4-coumarate CoA-ligase (4CL), cinnamoyl-CoA reductase (CCR), cinnamyl alcohol dehydrogenase (CAD) of the lignin biosynthetic pathway, from ryegrass (*Lolium*) and fescue (*Festuca*). The present invention also relates to regulatory elements, promoters capable of causing expression of exogenous genes in plants, wherein the regulatory elements are from the genes for caffeic acid Omethyl transferase (OMT), 4CL, CCR or CAD. The invention also relates to vectors including the nucleic acids and regulatory elements of the invention, plant cells, plants, plant seeds and other plant parts transformed with the regulatory elements, nucleic acids and vectors and methods using the nucleic acids, regulatory elements and vectors.

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MODIFICATION OF LIGNIN BIOSYNTHESIS

The present invention relates to the modification of lignin biosynthesis in plants and, more particularly, to enzymes involved in the lignin biosynthetic pathway and nucleic acids encoding such enzymes.

The present invention also relates to a regulatory element and, more particularly, to a promoter capable of causing expression of an exogenous gene in plant cells, such as a gene encoding an enzyme involved in the lignin biosynthetic pathway in plants.

The invention also relates to vectors including the nucleic acids and regulatory elements of the invention, plant cells, plants, seeds and other plant parts transformed with the regulatory elements, nucleic acids and vectors, and methods of using the nucleic acids, regulatory elements and vectors.

Lignins are complex phenolic polymers that strengthen plant cell walls against mechanical and chemical degradation. The process of lignification typically occurs during secondary thickening of the walls of cells with structural, conductive or defensive roles. Three monolignol precursors, sinapyl, coniferyl and *p*-coumaryl alcohol combine by dehydrogenative polymerisation to produce respectively the syringyl(S), guaiacyl(G) and hydroxyl(H) subunits of the lignin polymer, which can also become linked to cell-wall polysaccharides through the action of peroxidases and other oxidative isozymes. In grasses, biosynthesis of the monolignol precursors is a multistep process beginning with the aromatic amino-acids phenylalanine and tyrosine. It is the final two reduction/ dehydrogenation steps of the pathway, catalysed by Cinnamoyl CoA Reductase (CCR) and Cinnamyl Alcohol Dehydrogenase (CAD) that are considered to be specific to lignin biosynthesis. The proportions of monolignols incorporated into the lignin polymer vary depending on plant species, tissue, developmental stage and sub-cellular location.

Caffeic acid *O*-methyl transferase (OMT), 4 coumarate CoA-ligase (4CL), cinnamoyl-CoA reductase (CCR) and cinnamyl alcohol dehydrogenase

(CAD) are key enzymes involved in lignin biosynthesis.

Worldwide permanent pasture is estimated to cover 70% of agriculturally cultivated area. Ryegrasses (*Lolium* spp.) together with the closely related fescues (*Festuca* spp.) are of significant value in temperate grasslands. The commercially most important ryegrasses are Italian or annual ryegrass (*L. multiflorum* Lam.) and perennial ryegrass (*L. perenne* L.). They are the key forage species in countries where livestock production is an intensive enterprise, such as the Netherlands, United Kingdom and New Zealand. The commercially most important fescues are tall fescue (*F. anundinacea* Schreb.), meadow fescue (*F. pratensis*) and red fescue (*F. rubra*).

Perennial ryegrass (*Lolium perenne* L.) is the major grass species sown in temperate dairy pastures in Australia, and the key pasture grass in temperate climates throughout the world. A marked decline of the feeding value of grasses is observed in temperate pastures of Australia during late spring and early summer, where the nutritive value of perennial ryegrass based pasture is often insufficient to meet the metabolic demands of lactating dairy cattle. Perennial ryegrass is also an important turf grass.

Grass and legume *in vitro* dry matter digestibility has been negatively correlated with lignin content. In addition, natural mutants of lignin biosynthetic enzymes in maize, sorghum and pearl millet that have higher rumen digestibility have been characterised as having lower lignin content and altered S/G subunit ratio. Thus, lignification of plant cell walls is the major factor identified as responsible for lowering digestibility of forage tissues as they mature.

It would be desirable to have methods of altering lignin biosynthesis in plants, including grass species such as ryegrasses and fescues, by reducing the activity of key biosynthetic enzymes in order to reduce lignin content and/or alter lignin composition for enhancing dry matter digesitibility and improving herbage quality. However, for some applications it may be desirable to

enhance lignin biosynthesis to increase lignin content and/or alter lignin composition, for example to increase mechanical strength of wood, to increase mechanical strength of turf grasses, to reduce plant height and reduce lodging or improve disease resistance.

While nucleic acid sequences encoding some of the enzymes involved in the lignin biosynthetic pathway have been isolated for certain species of plants, there remains a need for materials useful in the modification of lignin biosynthesis in plants, particularly grass species such as ryegrasses and fescues.

Other phenotypic traits which may be improved by transgenic manipulation of plants include disease resistance, mineral content, nutrient quality and drought tolerance.

However, transgenic manipulation of phenotypic traits in plants requires the availability of regulatory elements capable of causing the expression of exogenous genes in plant cells.

It is an object of the present invention to overcome, or at least alleviate, one or more of the difficulties or deficiencies associated with the prior art.

In one aspect, the present invention provides substantially purified or isolated nucleic acids or nucleic acid fragments encoding the following enzymes from a ryegrass (*Lolium*) or fescue (*Festuca*) species: 4 coumarate CoA-ligase (4CL), cinnamoyl-CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD).

The ryegrass *(Lolium)* or fescue *(Festuca)* species may be of any suitable type, including Italian or annual ryegrass, perennial ryegrass, tall fescue, meadow fescue and red fescue. Preferably the ryegrass or fescue species is a ryegrass, more preferably perennial ryegrass *(Lolium perenne)*.

The nucleic acid or nucleic acid fragment may be of any suitable type

and includes DNA (such as cDNA or genomic DNA) and RNA (such as mRNA) that is single- or double- stranded, optionally containing synthetic, non-natural or altered nucleotide bases, and combinations thereof.

The term "isolated" means that the material is removed from its original environment (eg. the natural environment if it is naturally occurring). For example, a naturally occurring nucleic acid present in a living plant is not isolated, but the same nucleic acid separated from some or all of the coexisting materials in the natural system, is isolated. Such nucleic acids could be part of a vector and/or such nucleic acids could be part of a composition, and still be isolated in that such a vector or composition is not part of its natural environment.

In a preferred embodiment of this aspect of the invention, the substantially purified or isolated nucleic acid or nucleic acid fragment encoding 4CL includes a nucleotide sequence selected from the group consisting of (a) sequences shown in Figures 2, 3 and 4 hereto (Sequence ID Nos: 1, 3 and 5; respectively) (b) complements of the sequences shown in Figures 2, 3 and 4 hereto (Sequence ID Nos: 1, 3 and 5, respectively); (c) sequences antisense to the sequences recited in (a) and (b); and (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c).

In a further preferred embodiment of this aspect of the invention, the substantially purified or isolated nucleic acid or nucleic acid fragment encoding CCR includes a nucleotide sequence selected from the group consisting of (a) the sequence shown in Figure 10 hereto (Sequence ID No: 7); (b) the complement of the sequence shown in Figure 10 hereto (Sequence ID No: 7); (c) sequences antisense to the sequences recited in (a) and (b); and (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c).

In a still further preferred embodiment of this aspect of the invention, the substantially purified or isolated nucleic acid or nucleic acid fragment encoding CAD includes a nucleotide sequence selected from the group consisting of (a)

the sequences shown in Figures 13, 14, 26 and 27 hereto (Sequence ID Nos: 9, 11, 14 and 16, respectively); (b) complements of the sequences shown in Figures 13, 14, 26 and 27 hereto (Sequence ID Nos: 9, 11, 14 and 16, respectively); (c) sequences antisense to the sequences recited in (a) and (b); and (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c).

By "functionally active" is meant that the fragment or variant (such as an analogue, derivative or mutant) is capable of modifying lignin biosynthesis in a plant. Such variants include naturally occurring allelic variants and non-naturally occurring variants. Additions, deletions, substitutions and derivatizations of one or more of the nucleotides are contemplated so long as the modifications do not result in loss of functional activity of the fragment or variant. Preferably the functionally active fragment or variant has at least approximately 80% identity to the relevant part of the above mentioned sequence, more preferably at least approximately 90% identity, most preferably at least approximately 95% identity. Such functionally active variants and fragments include, for example, those having nucleic acid changes which result in conservative amino acid substitutions of one or more residues in the corresponding amino acid sequence. Preferably the fragment has a size of at least 10 nucleotides, more preferably at least 15 nucleotides, most preferably at least 20 nucleotides.

In a second aspect of the present invention there is provided a vector including a nucleic acid or nucleic acid fragment according to the present invention.

In a preferred embodiment of this aspect of the invention, the vector may include a regulatory element such as a promoter, a nucleic acid or nucleic acid fragment according to the present invention and a terminator; said regulatory element, nucleic acid or nucleic acid fragment and terminator being operatively linked.

By "operatively linked" is meant that said regulatory element is capable

of causing expression of said nucleic acid or nucleic acid fragment in a plant cell and said terminator is capable of terminating expression of said nucleic acid or nucleic acid fragment in a plant cell. Preferably, said regulatory element is upstream of said nucleic acid or nucleic acid fragment and said terminator is downstream of said nucleic acid or nucleic acid fragment.

The vector may be of any suitable type and may be viral or non-viral. The vector may be an expression vector. Such vectors include chromosomal, non-chromosomal and synthetic nucleic acid sequences, eg. derivatives of plant viruses; bacterial plasmids; derivatives of the Ti plasmid from *Agrobacterium tumefaciens*; derivatives of the Ri plasmid from *Agrobacterium rhizogenes;* phage DNA; yeast artificial chromosomes; bacterial artificial chromosomes; binary bacterial artificial chromosomes; vectors derived from combinations of plasmids and phage DNA. However, any other vector may be used as long as it is replicable or integrative or viable in the plant cell.

The regulatory element and terminator may be of any suitable type and may be endogenous to the target plant cell or may be exogenous, provided that they are functional in the target plant cell.

Preferably the regulatory element is a promoter. A variety of promoters which may be employed in the vectors of the present invention are well known to those skilled in the art. Factors influencing the choice of promoter include the desired tissue specificity of the vector, and whether constitutive or inducible expression is desired and the nature of the plant cell to be transformed (eg. monocotyledon or dicotyledon). Particularly suitable promoters include the Cauliflower Mosaic Virus 35S (CaMV 35S) promoter, the maize Ubiquitin promoter, the rice Actin promoter, and ryegrass endogenous OMT, 4CL, CCR or CAD promoters.

A variety of terminators which may be employed in the vectors of the present invention are also well known to those skilled in the art. The terminator may be from the same gene as the promoter sequence or a different gene. Particularly suitable terminators are polyadenylation signals, such as the

CaMV 35S polyA and other terminators from the nopaline synthase (*nos*) and the octopine synthase (*ocs*) genes.

The vector, in addition to the regulatory element, the nucleic acid or nucleic acid fragment of the present invention and the terminator, may include further elements necessary for expression of the nucleic acid or nucleic acid fragment, in different combinations, for example vector backbone, origin of replication (ori), multiple cloning sites, spacer sequences, enhancers, introns (such as the maize Ubiquitin Ubi intron), antibiotic resistance genes and other selectable marker genes [such as the neomycin phosphotransferase (*npt2*) gene, the hygromycin phosphotransferase (*hph*) gene, the phosphinothricin acetyltransferase (*bar* or *pat*) gene], and reporter genes (such as beta-glucuronidase (GUS) gene (*gusA*)]. The vector may also contain a ribosome binding site for translation initiation. The vector may also include appropriate sequences for amplifying expression.

As an alternative to use of a selectable marker gene to provide a phenotypic trait for selection of transformed host cells, the presence of the vector in transformed cells may be determined by other techniques well known in the art, such as PCR (polymerase chain reaction), Southern blot hybridisation analysis, histochemical GUS assays, northern and Western blot hybridisation analyses.

Those skilled in the art will appreciate that the various components of the vector are operatively linked, so as to result in expression of said nucleic acid or nucleic acid fragment. Techniques for operatively linking the components of the vector of the present invention are well known to those skilled in the art. Such techniques include the use of linkers, such as synthetic linkers, for example including one or more restriction enzyme sites.

The vectors of the present invention may be incorporated into a variety of plants, including monocotyledons (such as grasses from the genera *Lolium, Festuca, Paspalum, Pennisetum, Panicum* and other forage and turf grasses, corn, oat, sugarcane, wheat and barley), dicotyledons (such as arabidopsis,

tobacco, legumes, alfalfa, oak, eucalyptus, maple, canola, soybean and chickpea) and gymnosperms. In a preferred embodiment, the vectors are used to transform monocotyledons, preferably grass species such as ryegrasses (*Lolium* species) and fescues (*Festuca* species), more preferably perennial ryegrass *(Lolium perenne)* including forage and turf type cultivars.

Techniques for incorporating the vectors of the present invention into plant cells (for example by transduction, transfection or transformation) are well known to those skilled in the art. Such techniques include *Agrobacterium* mediated introduction, electroporation to tissues, cells and protoplasts, protoplast fusion, injection into reproductive organs, injection into immature embryos and high velocity projectile introduction to cells, tissues, calli, immature and mature embryos. The choice of technique will depend largely on the type of plant to be transformed.

Cells incorporating the vector of the present invention may be selected, as described above, and then cultured in an appropriate medium to regenerate transformed plants, using techniques well known in the art. The culture conditions, such as temperature, pH and the like, will be apparent to the person skilled in the art. The resulting plants may be reproduced, either sexually or asexually, using methods well known in the art, to produce successive generations of transformed plants.

In a further aspect of the present invention there is provided a plant cell, plant, plant seed or other plant part, including, eg transformed with, a vector of the present invention.

The plant cell, plant, plant seed or other plant part may be from any suitable species, including monocotyledons, dicotyledons and gymnosperms. In a preferred embodiment the plant cell, plant, plant seed or other plant part may be from a monocotyledon, preferably a grass species, more preferably a ryegrass (*Lolium* species) or fescue (*Festuca* species), even more preferably a ryegrass, most preferably perennial ryegrass, including forage- and turf-type cultivars.

The present invention also provides a plant, plant seed or other plant part derived from a plant cell of the present invention.

The present invention also provides a plant, plant seed or other plant part derived from a plant of the present invention.

In a further aspect of the present invention there is provided a method of modifying lignin biosynthesis in a plant, said method including introducing into said plant an effective amount of a nucleic acid or nucleic acid fragment and/or a vector according to the present invention.

By "an effective amount" is meant an amount sufficient to result in an identifiable phenotypic trait in said plant, or a plant, plant seed or other plant part derived therefrom. Such amounts can be readily determined by an appropriately skilled person, taking into account the type of plant, the route of administration and other relevant factors. Such a person will readily be able to determine a suitable amount and method of administration. See, for example, Maniatis et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, the entire disclosure of which is incorporated herein by reference.

Using the methods and materials of the present invention, plant lignin biosynthesis may be increased, decreased or otherwise modified relative to an untransformed control plant. It may be increased or otherwise modified, for example, by incorporating additional copies of a sense nucleic acid or nucleic acid fragment of the present invention. It may be decreased, for example, by incorporating an antisense nucleic acid or nucleic acid fragment of the present invention. In addition, the number of copies of genes encoding for different enzymes in the lignin biosynthetic pathway may be manipulated to modify the relative amount of each monolignol synthesized, thereby leading to the formation of lignin having altered composition.

In a still further aspect of the present invention there is provided use of a nucleic acid or nucleic acid fragment according to the present invention, and/or

nucleotide sequence information thereof, and/or single nucleotide polymorphisms thereof, as a molecular genetic marker.

More particularly, nucleic acids or nucleic acid fragments according to the present invention, and/or nucleotide sequence information thereof, and/or single nucleotide polymorphisms thereof, may be used as a molecular genetic marker for qualitative trait loci (QTL) tagging, mapping, DNA fingerprinting and in marker assisted selection, and may be used as candidate genes or perfect markers, particularly in ryegrasses and fescues. Even more particularly, nucleic acids or nucleic acid fragments according to the present invention, and/or nucleotide sequence information thereof, may be used as molecular genetic markers in forage and turf grass improvement, eg. tagging QTLs for dry matter digestibility, herbage quality, mechanical stress tolerance, disease resistance, insect pest resistance, plant stature and leaf and stem colour.

In a still further aspect of the present invention there is provided a substantially purified or isolated polypeptide from a ryegrass *(Lolium)* or fescue *(Fustuca)* species, selected from the group consisting of the enzymes 4CL, CCR and CAD.

The ryegrass *(Lolium)* or fescue *(Festuca)* species may be of any suitable type, including Italian or annual ryegrass, perennial ryegrass, tall fescue, meadow fescue and red fescue. Preferably the species is a ryegrass, more preferably perennial ryegrass *L. perenne)*.

In a preferred embodiment of this aspect of the invention, the substantially purified or isolated enzyme 4CL includes an amino acid sequence selected from the group consisting of sequences shown in Figures 2, 3 and 4 hereto (Sequence ID Nos: 2, 4 and 6, respectively); and functionally active fragments and variants thereof.

In a further preferred embodiment of this aspect of the invention, the substantially purified or isolated enzyme CCR includes an amino acid sequence selected from the group consisting of the sequence shown in Figure

10 hereto (Sequence ID No: 8); and functionally active fragments and variants thereof.

In a still further preferred embodiment of this aspect of the invention, the substantially purified or isolated enzyme CAD includes an amino acid sequence selected from the group consisting of the sequence shown in Figures 13, 14, 26 and 27 hereto (Sequence ID Nos: 10, 12, 15 and 17, respectively); and functionally active fragments and variants thereof.

By "functionally active" in this context is meant that the fragment or variant has one or more of the biological properties of the enzymes 4CL, CCR and CAD, respectively. Additions, deletions, substitutions and derivatizations of one or more of the amino acids are contemplated so long as the modifications do not result in loss of functional activity of the fragment or variant. Preferably the fragment or variant has at least approximately 60% identity to the relevant part of the above mentioned sequence, more preferably at least approximately 80% identity, most preferably at least approximately 90% identity. Such functionally active variants and fragments include, for example, those having conservative amino acid substitutions of one or more residues in the corresponding amino acid sequence. Preferably the fragment has a size of at least 10 amino acids, more preferably at least 15 amino acids, most preferably at least 20 amino acids.

In a further embodiment of this aspect of the invention, there is provided a polypeptide recombinantly produced from a nucleic acid or nucleic acid fragment according to the present invention. Techniques for recombinantly producing polypeptides are well known to those skilled in the art.

In a still further aspect of the present invention there is provided a lignin or modified lignin substantially or partially purified or isolated from a plant, plant seed or other plant part of the present invention.

Such lignins may be modified from naturally occurring lignins in terms of the length, the degree of polymerisation (number of units), degree of branching

and/or nature of linkages between units.

In a still further aspect, the present invention provides an isolated regulatory element capable of causing expression of an exogenous gene in plant cells. Preferably the regulatory element is isolated from a nucleic acid or nucleic acid fragment encoding OMT, 4CL, CCR or CAD.

The regulatory element may be a nucleic acid molecule, including DNA (such as cDNA or genomic DNA) and RNA (such as mRNA) that is single- or double- stranded, optionally containing synthetic, non-natural or altered nucleotide bases, and combinations thereof.

Preferably the regulatory element includes a promoter, more preferably an *O*-methyltransferase promoter, even more preferably an *O*-methyltransferase promoter from a ryegrass (*Lolium*) or fescue (*Festuca*) species, more preferably a ryegrass, most preferably perennial ryegrass (*Lolium perenne*).

In a particularly preferred embodiment of this aspect of the invention, the regulatory element includes a promoter from the caffeic acid *O*-methyltransferase gene corresponding to the cDNA homologue *Lp*OMT1 from perennial ryegrass.

Preferably the regulatory element includes a nucleotide sequence including the first approximately 4630 nucleotides of the sequence shown in Figure 18 hereto (Sequence ID No: 13); or a functionally active fragment or variant thereof.

By "functionally active" in this context is meant that the fragment or variant (such as an analogue, derivative or mutant) is capable of causing expression of a transgene in plant cells. Such variants include naturally occurring allelic variants and non-naturally occurring variants. Additions, deletions, substitutions and derivatizations of one or more of the nucleotides are contemplated so long as the modifications do not result in loss of functional

activity of the regulatory element. Preferably the functionally active fragment or variant has at least approximately 80% identity to the relevant part of the above sequence, more preferably at least approximately 90% identity, most preferably at least approximately 95% identity. Preferably the fragment has a size of at least 100 nucleotides, more preferably at least 150 nucleotides, most preferably at least 200 nucleotides.

In a particularly preferred embodiment of this aspect of the invention, the regulatory element includes a nucleotide sequence selected from the group consisting of:

Nucleotides – 4581 to –1

Nucleotides -4285 to –1

Nucleotides –4020 to –1

Nucleotides –2754 to –1

Nucleotides – 1810 to –1

Nucleotides –831 to –1

Nucleotides –560 to –1

Nucleotides –525 to –1

Nucleotides –274 to –1

Nucleotides –21 to -1

of Figure 18 hereto (Sequence ID No: 13);

or a functionally active fragment or variant thereof.

In another preferred embodiment the regulatory element includes a 4 coumarate-CoA ligase promoter, even more preferably a 4 coumarate-CoA ligase promoter from a ryegrass (*Lolium*) or fescue (*Festuca*) species, more preferably a ryegrass, most preferably perennial ryegrass (*Lolium perenne*).

In a particularly preferred embodiment of this aspect of the invention, the regulatory element includes a promoter from the 4 coumarate-CoA ligase gene corresponding to the cDNA homologue *Lp*4CL2 from perennial ryegrass.

Preferably the regulatory element includes a nucleotide sequence including the first approximately 2206 nucleotides of the sequence shown in Figure 38 hereto (Sequence ID No: 17); or a functionally active fragment or variant thereof.

By "functionally active" in this context is meant that the fragment or variant (such as an analogue, derivative or mutant) is capable of causing expression of a transgene in plant cells. Such variants include naturally occurring allelic variants and non-naturally occurring variants. Additions, deletions, substitutions and derivatizations of one or more of the nucleotides are contemplated so long as the modifications do not result in loss of functional activity of the regulatory element. Preferably the functionally active fragment or variant has at least approximately 80% identity to the relevant part of the above sequence, more preferably at least approximately 90% identity, most preferably at least approximately 95% identity. Preferably the fragment has a size of at least 100 nucleotides, more preferably at least 150 nucleotides, most preferably at least 200 nucleotides.

In a particularly preferred embodiment of this aspect of the invention, the regulatory element includes a nucleotide sequence selected from the group consisting of:

Nucleotides – 2206 to –1

Nucleotides -1546 to –1

Nucleotides –1186 to –1

Nucleotides –406 to –1

Nucleotides – 166 to –1

of Figure 38 hereto (Sequence ID No: 17);

or a functionally active fragment or variant thereof.

In another preferred embodiment the regulatory element includes a cinnamoyl-CoA reductase promoter, even more preferably a cinnamoyl-CoA reductase promoter from a ryegrass (*Lolium*) or fescue (*Festuca*) species, more preferably a ryegrass, most preferably perennial ryegrass (*Lolium perenne*).

In a particularly preferred embodiment of this aspect of the invention, the regulatory element includes a promoter from the cinnamoyl-CoA reductase gene corresponding to the *Lp*CCR1 cDNA from perennial ryegrass.

Preferably the regulatory element includes a nucleotide sequence including the first approximately 6735 nucleotides of the sequence shown in Figure 39 hereto (Sequence ID No: 18); or a functionally active fragment or variant thereof.

By "functionally active" in this context is meant that the fragment or variant (such as an analogue, derivative or mutant) is capable of causing expression of a transgene in plant cells. Such variants include naturally occurring allelic variants and non-naturally occurring variants. Additions, deletions, substitutions and derivatizations of one or more of the nucleotides are contemplated so long as the modifications do not result in loss of functional activity of the regulatory element. Preferably the functionally active fragment or variant has at least approximately 80% identity to the relevant part of the above sequence, more preferably at least approximately 90% identity, most preferably at least approximately 95% identity. Preferably the fragment has a size of at least 100 nucleotides, more preferably at least 150 nucleotides, most preferably at least 200 nucleotides.

In a particularly preferred embodiment of this aspect of the invention, the regulatory element includes a nucleotide sequence selected from the group consisting of:

Nucleotides – 6735 to –1

Nucleotides -5955 to –1

Nucleotides –5415 to –1

Nucleotides –4455 to –1

Nucleotides – 4035 to –1

Nucleotides –3195 to –1

Nucleotides –2595 to –1

Nucleotides –1755 to –1

Nucleotides –1275 to –1

Nucleotides –495 to –1

Nucleotides –255 to –1

Nucleotides –75 to –1

of Figure 39 hereto (Sequence ID No: 18);

or a functionally active fragment or variant thereof.

By an "exogenous gene" is meant a gene not natively linked to said regulatory element. In certain embodiments of the present invention the exogenous gene is also not natively found in the relevant plant or plant cell.

The exogenous gene may be of any suitable type. The exogenous gene may be a nucleic acid such as DNA (e.g. cDNA or genomic DNA) or RNA (e.g. mRNA), and combinations thereof. The exogenous gene may correspond to a target gene, for example a gene capable of influencing disease resistance, herbage digestibility, nutrient quality, mineral content or drought tolerance or be a fragment or variant (such as an analogue, derivative or mutant) thereof

which is capable of modifying expression of said target gene. Such variants include nucleic acid sequences which are antisense to said target gene or an analogue, derivative, mutant or fragment thereof. The transgene may code for a protein or RNA sequence depending the target condition and whether down or up-regulation of gene expression is required. Preferably, the target gene is selected from exogenous coding sequences coding for mRNA for a protein, this protein may be of bacterial origin (such as enzymes involved in cell wall modification and cell wall metabolism, cytokinin biosynthesis), or eukaryotic origin (such as pharmaceutically active polypeptides) or of plant origin (such as enzymes involved in the synthesis of phenolic compounds, cell wall metabolism, sugar metabolism, lignin biosynthesis). Preferably, the target gene is selected from the group comprising *O*-methyltransferase, 4 coumarate CoA-ligase, cinnamoyl CoA reductase, cinnamyl alcohol dehydrogenase, cinnamate 4 hydroxylase, phenolase, laccase, peroxidase, coniferol glucosyl transferase, coniferin beta-glucosidase, phenylalanine ammonia lyase, ferulate 5-hydroxylase, chitinase, glucanase, isopentenyltransferase, xylanase.

The plant cells, in which the regulatory element of the present invention is capable of causing expression of an exogenous gene, may be of any suitable type. The plant cells may be from monocotyledons (such as grasses from the genera *Lolium, Festuca, Paspalum, Pennisetum, Panicum* and other forage and turf grasses, corn, grains, oat, sugarcane, wheat and barley), dicotyledons (such as arabidopsis, tobacco, legumes, alfalfa, oak, eucalyptus and maple) and gymnosperms. Preferably the plant cells are from a monocotyledon, more preferably a grass species such as a ryegrass (*Lolium*) or fescue (*Festuca*) species, even more preferably a ryegrass, most preferably perennial ryegrass (*Lolium perenne*).

The regulatory element according to the present invention may be used to express exogenous genes to which it is operatively linked in the production of transgenic plants.

Accordingly, in a further aspect of the present invention there is provided a vector including a regulatory element according to the present

invention.

In a preferred embodiment of this aspect of the invention, the vector may include a regulatory element according to the present invention, an exogenous gene as hereinbefore described, and a terminator; said regulatory element, exogenous gene and terminator being operatively linked, such that said regulatory element is capable of causing expression of said exogenous gene in plant cells. Preferably, said regulatory element is upstream of said exogenous gene and said terminator is downstream of said exogenous gene.

The vector may be of any suitable type and may be viral or non-viral. The vector may be an expression vector. Such vectors include chromosomal, non-chromosomal and synthetic nucleic acid sequences, eg. derivatives of plant viruses; bacterial plasmids; derivatives of the Ti plasmid from *Agrobacterium tumefaciens*; derivatives of the Ri plasmid from *Agrobacterium rhizogenes;* phage DNA; yeast artificial chromosomes; bacterial artificial chromosomes; binary bacterial artificial chromosomes; vectors derived from combinations of plasmids and phage DNA. However, any other vector may be used as long as it is replicable on integrative or viable in the plant cell.

The terminator may be of any suitable type and includes for example polyadenylation signals, such as the Cauliflower Mosaic Virus 35S polyA (CaMV 35S polyA) and other terminators from the nopaline synthase (*nos*) and the octopine synthase (*ocs*) genes.

The vector, in addition to the regulatory element, the exogenous nucleic acid and the terminator, may include further elements necessary for expression of the nucleic acid, in different combinations, for example vector backbone, origin of replication (ori), multiple cloning sites, spacer sequences, enhancers, introns (such as the maize Ubiquitin Ubi intron), antibiotic resistance genes and other selectable marker genes [such as the neomycin phosphotransferase (*npt2*) gene, the hygromycin phosphotransferase (*hph*) gene, the phosphinothricin acetyltransferase (*bar* or *pat*) gene], and reporter genes (such as beta-glucuronidase (GUS) gene (*gusA*)]. The vector may also

contain a ribosome binding site for translation initiation. The vector may also include appropriate sequences for amplifying expression.

The regulatory element of the present invention may also be used with other full promoters or partial promoter elements.

As an alternative to use of a selectable marker gene to provide a phenotypic trait for selection of transformed host cells, the presence of the vector in transformed cells may be determined by other techniques well known in the art, such as PCR (polymerase chain reaction), Southern blot hybridisation analysis, histochemical GUS assays, northern and Western blot hybridisation analyses.

Those skilled in the art will appreciate that the various components of the vector are operatively linked, so as to result in expression of said transgene. Techniques for operatively linking the components of the vector of the present invention are well known to those skilled in the art. Such techniques include the use of linkers, such as synthetic linkers, for example including one or more restriction sites.

The vectors of the present invention may be incorporated into a variety of plants, including monocotyledons, dicotyledons and gymnosperms. In a preferred embodiment the vectors are used to transform monocotyledons, preferably grass species such as ryegrasses (*Lolium* species) and fescues (*Festuca* species), more preferably perennial ryegrass (*Lolium perenne*) including forage- and turf- type cultivars.

Techniques for incorporating the vectors of the present invention into plant cells (for example by transduction, transfection or transformation) are well known to those skilled in the art. Such techniques include *Agrobacterium* mediated introduction, electroporation to tissues, cells and protoplasts, protoplast fusion, injection into reproductive organs, injection into immature embryos and high velocity projectile introduction to cells, tissues, calli, immature and mature embryos. The choice of technique will depend largely

on the type of plant to be transformed.

Cells incorporating the vector of the present invention may be selected, as described above, and then cultured in an appropriate medium to regenerate transformed plants, using techniques well known in the art. The culture conditions, such as temperature, pH and the like, will be apparent to the person skilled in the art. The resulting plants may be reproduced, either sexually or asexually, using methods well known in the art, to produce successive generations of transformed plants.

In a further aspect of the present invention there is provided a plant cell, plant, plant seed or other plant part, including, eg. transformed with, a vector of the present invention.

The plant cell, plant, plant seed or other plant part may be from any suitable species, including monocotyledons, dicotyledons and gymnosperms. In a preferred embodiment the plant cell, plant, plant seed or other plant part is from a monocotyledon, preferably a grass species, more preferably a ryegrass (*Lolium* species) or fescue (*Festuca* species), even more preferably perennial ryegrass (*Lolium perenne*), including forage- and turf-type cultivars.

The present invention also provides a plant, plant seed, or other plant part derived from a plant cell of the present invention.

The present invention also provides a plant, plant seed or other plant part derived from a plant of the present invention.

In a still further aspect of the present invention there is provided a recombinant plant genome including a regulatory element according to the present invention.

In a preferred embodiment of this aspect of the invention the recombinant plant genome further includes an exogenous gene operatively linked to said regulatory element.

In a further aspect of the present invention there is provided a method for expressing an exogenous gene in plant cells, said method including introducing into said plant cells an effective amount of a regulatory element and/or a vector according to the present invention.

By "an effective amount" is meant an amount sufficient to result in an identifiable phenotypic change in said plant cells or a plant, plant seed or other plant part derived therefrom. Such amounts can be readily determined by an appropriately skilled person, taking into account the type of plant cell, the route of administration and other relevant factors. Such a person will readily be able to determine a suitable amount and method of administration. See, for example, Maniatis et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, the entire disclosure of which is incorporated herein by reference.

The present invention will now be more fully described with reference to the accompanying Examples and drawings. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

In the Figures

Figure 1 shows plasmid maps of the three cDNAs encoding perennial ryegrass 4CL homologues.

Figure 2 shows the nucleotide (Sequence ID No: 1) and amino acid (Sequence ID No: 2) sequences of *Lp*4CL1.

Figure 3 shows the nucleotide (Sequence ID No: 3) and amino acid (Sequence ID No: 4) sequences of *Lp*4CL2.

Figure 4 shows the nucleotide (Sequence ID No: 5) and amino acid (Sequence ID No: 6) sequences of *Lp*4CL3.

Figure 5 shows amino acid sequence alignment of deduced proteins encoded by *Lp*4CL1 (Sequence ID No: 2), *Lp*4CL2 (Sequence ID No: 4) and *Lp*4CL3 (Sequence ID No: 6).

Figure 6 shows northern hybridisation analysis of developing perennial ryegrass using *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 as hybridisation probes. SR: roots from seedlings (3-5 d post-germination), SS: shoots from seedlings (3-5 d post-germination), ML: leaves from 12-week-old plants, MS: stems from 12-week-old plants. Blots were washed in 0.2 X SSPE, 0.1 % SDS at 65 °C. *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 do not cross hybridise at this stringency. Sizes are given in kb.

Figure 7 shows northern hybridisation analysis showing the time course of expression of 4CL mRNA in wounded perennial ryegrass leaves. Sizes are given in kb.

Figure 8 shows genomic Southern hybridisation analysis using *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 as hybridisation probes. 10 µg of digested perennial ryegrass genomic DNA or 20 µg of digested tall fescue genomic DNA were separated on a 1.0 % agarose gel, transferred to Hybond N^+ membranes and then hybridised with ^{32}P labelled *Lp*4CL1, *Lp*4CL2 or *Lp*4CL3 probes. The ryegrass *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 genes reveal homologous sequences in tall fescue and indicate that the ryegrass 4CL genes can be used to isolate and to manipulate the expression of the tall fescue (*Festuca arundinacea*) 4CL genes.

Figure 9 shows restriction map of *Lp*CCR1. An *L. perenne* seedling cDNA library constructed in Uni-ZAP™ (Stratagene) was screened in a solution containing 10xPIPES, 50% deionised formamide and 10% SDS at 42°C. Filters were washed at room temperature, three times in 0.1% SDS, 2× SSPE and then twice in 0.1% SDS, 0.2× SSPE. The location of the probe used for northern and Southern hybridisation analyses is indicated by the black line labelled *Lp*CCR531.

Figure 10 shows the nucleotide (Sequence ID No: 7) and amino acid (Sequence ID No: 8) sequences of *Lp*CCR1.

Figure 11 shows Southern hybridisation analysis of DNA from double haploid (DH) perennial ryegrass using *Lp*CCR1 as hybridisation probe. 10µg of DH genomic DNA was digested with DraI, BamHI, EcoRI, EcoRV, HindIII or XbaI, separated on a 1% agarose gel and then capillary blotted onto nylon membrane (Amersham Hybond-N). The membrane was probed with the digoxigenin (DIG) labelled LpCCR531 fragment at 25ng/ml in the hybridisation solution. Hybridisation was in 4x SSC, 50% formamide, 0.1% N-Lauroyl-sarcosine, 0.02% SDS, 2% Blocking solution at 42°C. The membrane was washed twice for five minutes in 2x SSC, 0.1% SDS at room temperature, then twice for fifteen minutes in 0.5x SSC, 0.1%SDS at 68°C. Molecular weight was determined by comparison to a DIG-labelled marker (Roche Molecular Biochemicals).

Figure 12 shows northern hybridisation analysis of RNA samples from different organs and developmental stages of perennial ryegrass using *Lp*CCR1 probe. Roots from seedlings (3-5 d post-germination), shoots from seedlings (3-5 d post-germination), roots from seedlings (7-10 d post-germination), leaves from seedlings (7-10 d post-germination), roots from 6 and 10 week old plants, leaves from 6 and 10 week old plants, stems from 6 and 10 week old plants, whole seedling from 11 day old *Phalaris* and 7 day old *Festuca*.

Total RNA was isolated using Trizol (GibcoBRL) and 15 µg was separated on a 1.2% Agarose gel containing 6% formamide and then capillary blotted onto nylon membrane (Amersham Hybond-N). The membrane was stained with 0.2% methylene blue/0.3M sodium acetate to visualise the marker and ensure that RNA was evenly loaded. 50 ng LpCCR531 was random-labelled with ^{32}P-dCTP (Amersham Megaprime) and hybridisation conditions were 4x SSC, 50% formamide, 0.5% SDS, 5x denhardt solution, 5% dextrane sulphate, 0.1% Herring sperm DNA at 42°C over-night. The ryegrass *Lp*CCR1

gene reveal homologous transcripts in tall fescue and *Phalaris*, thus indicating that the ryegrass CCR gene can be used to manipulate the expression of the tall fescue (*Festuca arundinacea*) and *Phalaris* CCR endogenous genes.

Figure 13 shows the nucleotide (Sequence ID No: 9) and amino acid (Sequence ID No: 10) sequences of *Lp*CAD1.

Figure 14 shows the nucleotide (Sequence ID No: 11) and amino acid (Sequence ID No: 12) sequences of *Lp*CAD2.

Figure 15 shows a plasmid map of a cDNA clone encoding perennial ryegrass CAD homologue *Lp*CAD1.

Figure 16 shows northern hybridisation analysis of RNA samples from different organs and developmental stages of perennial ryegrass using A) *Lp*CAD1 and B) *Lp*CAD2 as hybridisation probes. Roots from seedlings 3-5 d post-germination, 7-10 d post-germination, 6 weeks and 10 weeks, Shoots from seedlings 3-5 d post-germination and 7-10 d post-germination, Leaves from 6 week old and 10 week old plants, stem tissue from 6 and 10 week old plants. RNA isolated from *Phalaris* and *Festuca* 11 and 7 day old seedlings. The ryegrass CAD genes reveal homologous transcripts in tall fescue and *Phalaris*, thus indicating that the ryegrass CAD gene can be used to manipulate the expression of the tall fescue and *Phalaris* CAD endogenous genes.

Figure 17 shows genomic Southern hybridisation analysis. 10 μg of perennial ryegrass genomic DNA digested with a range of restriction enzymes was separated on a 0.8% agarose gel, transferred to Hybond N and then hybridised with a DIG labelled A) *Lp*CAD1, and B) *Lp*CAD2 hybridisation probe.

Figure 18 shows the nucleotide sequence of the *LpOmt1* promoter (Sequence ID No: 13).

Figure 19 shows a plasmid map of plant transformation vector carrying the reporter β-glucuronidase (GUS) gene (*gus*A) under control of the perennial ryegrass *Lp*Omt1 promoter.

Figure 20 (upper image) shows PCR analysis of transgenic tobacco plants containing the *gus*A gene under the control of the perennial ryegrass *Lp*OMT1 promoter (upper figure). PCR reactions using *gusA*-specific primers were performed. Figure 20 (lower images) show histochemical GUS assays, demonstrating xylem-specific *gusA* expression (A and B) and *gusA* expression in glandular leaf trichomes (C and D) in transgenic tobacco plants containing the *gus*A gene under the control of the perennial ryegrass *Lp*OMT1 promoter.

Figure 21 shows the isolation of the *Lp*CCR1 genomic clone 1. A) Southern hybridization analysis of CCR genomic clone λLp6.1.1a digested with X*ba*I, *Nco*I, S*al*I, *Xho*I, X*ho*I/S*al*I DNA was separated on a 0.8% agarose gel, transferred to Hybond N and hybridized with a DIG labelled CCR1 probe. B) Map showing the genomic gene organisation of *Lp*CCR1 clone 1 based on sequence results. C) Comparison of plant CCR exon size and number in different plant species (*Lolium perenne, Lp., Eucalyptus gunni, Eg., Eucalyptus saligna, Es., Populus balsamifera, Pb.*)

Figure 22 shows the isolation of the *Lp*CCR1 genomic clone 2. A) Southern hybridization analysis of CCR genomic clone λLp6.1.1a digested with X*ba*I, N*co*I, S*al*I, X*ho*I, X*ho*I/S*al*I DNA was separated on a 0.8% agarose gel, transferred to Hybond N and hybridized with 200bp of the CCR1 promoter (Figure 21B). B) Map showing the promoter region of *Lp*CCR1 clone 2 based on sequence results.

Figure 23 shows the isolation of an *Lp*4CL genomic clone. A) Southern hybridisation analysis of 4CL genomic clone λ*Lp*4CL2 digested with BamHI, KpnI or SalI. DNA was separated on a 0.8% agarose gel, transferred to Hybond N and hybridized with a DIG labelled 4CL1 hybridisation probe. B) 10μl of a standard PCR reaction using forward and reverse oligonucleotides

designed to positions outlined on C). The PCR products were separated on a 0.8% agarose gel and stained with ethidium bromide. C) Map showing the genomic gene organisation of λ*Lp*4CL2 based on sequence and PCR results.

Figure 24 shows the isolation of an *Lp*4CL genomic clone. A) Southern hybridisation analysis of 4CL genomic clone λ*Lp*4CL2 digested with BamHI, KpnI, SalI. DNA was separated on a 0.8% agarose gel, transferred to Hybond N and hybridized with a DIG labelled 4CL1 probe. B) Map showing the genomic gene organisation of *Lp*4CL2 clone 1 and the promoter region of clone 2.

Figure 25 shows plasmid map of plant transformation vector carrying the *gusA* gene under control of the perennial ryegrass *Lp*4CL2 promoter (*Lp*4CL2::*gusA*).

Figure 26 shows nucleotide (Sequence ID No: 14) and amino acid (Sequence ID No: 15) sequences of genomic clone CAD2 cv Barlano (Intron 1 and first 111 bp of the coding region are missing).

Figure 27 shows nucleotide (Sequence ID No: 16) and amino acid (Sequence ID No:15) sequences of coding sequence deduced from genomic clone CAD2 cv Barlano (region in bold is missing from the genomic clone).

Figure 28 shows the isolation of *Lp*CAD2 genomic clone. A) Southern hybridization analysis of CAD genomic clone λ*Lp*CAD2 digested with BamHI, EcoRI, KpnI, SalI or XbaI. DNA was separated on a 0.8% agarose gel, transferred to Hybond N and hybridized with a DIG labelled CAD2 hybridisation probe. B) Map showing the genomic gene organisation of λ*Lp*CAD2 based on sequence results.

Figure 29 shows A) Sense and antisense *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 transformation vectors under control of the CaMV 35S promoter; B) Sense and antisense *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 transformation vectors under control

of the maize ubiquitin promoter.

Figure 30 shows A) Sense and antisense *Lp*CCR1 transformation vectors under control of the CaMV 35S promoter; B) Sense and antisense *Lp*CCR1 transformation vectors under control of the maize ubiquitin promoter.

Figure 31 shows A) Sense and antisense *Lp*CAD1 transformation vectors under control of the CaMV 35S promoter; B) Sense and antisense *Lp*CAD1 transformation vectors under control of the maize ubiquitin promoter.

Figure 32 shows molecular analysis of *Lp*4CL1-transgenic tobacco. A) Plasmid map of transformation vector carrying a chimeric sense *Lp*4CL1 gene. B) PCR analysis of independent transgenic tobacco clones using *Lp*4CL1 specific primers. C) Southern hybridization analysis of independent transgenic tobacco plants using an *Lp*4CL1 specific probe. D) Northern hybridization analysis of independent transgenic tobacco plants using an *Lp*4CL1 specific probe.

Figure 33 shows molecular analysis of *Lp*CCR1-transgenic tobacco. A) Plasmid map of transformation vectors carrying a chimeric sense and antisense *Lp*CCR1 gene. B) PCR analysis of independent sense transgenic tobacco clones using *Lp*CCR1 specific primers.

Figure 34 shows protocol for suspension culture-independent production of transgenic perennial ryegrass plants. A) Isolated zygotic embryos, plated on MSM5 medium, day 0; B) Embryogenic callus formation and proliferation, 6 - 8 weeks after embryo isolation; C) Embryogenic calli arranged on high osmotic MSM3Plus medium prior to biolistic transformation; D) Histochemical GUS assay showing GUS expressing foci 3 – 4 days post-bombardment of chimeric *gus*A gene; E) Selection of embryogenic calli on MSM3 medium containing 100 mg/l paromomycin (Pm), 2 weeks after microprojectile bombardment; F) Regeneration of Pm resistant shoots on MSK medium containing 100 mg/l Pm, 4 weeks after microprojectile bombardment; G) *In vitro* plant regeneration from PM resistant embryogenic calli, 6 weeks

after microprojectile bombardment; H) Transgenic perennial ryegrass plants 28 weeks after embryo isolation.

Figure 35 shows molecular analysis of transgenic perennial ryegrass plants carrying sense and antisense *Lp*Omt1 transgenes. Plasmid maps of vectors used for the co-transformation of perennial ryegrass embryogenic calli; pHP23 carrying a chimeric neomycin phosphotransferase (*npt2*) selectable marker gene; pUbiomt1 carrying a maize ubiquitin promoter driven sense *Lp*Omt1 gene; pUbitmo1 carrying a maize ubiquitin promoter driven antisense *Lp*Omt1 gene (top). PCR analysis using *npt2*-specific primers of 5 independent transgenic perennial ryegrass plants from biolistic transformation with sense and antisense *Lp*Omt1 vectors (upper centre). Southern hybridization analysis with an *omt*1 hybridization probe of 7 independent perennial ryegrass plants co-transformed with sense (lanes 1-3) and antisense (lanes 4-7) *Lp*Omt1 vectors (lower centre left). Southern hybridisation analysis with an *npt2* hybridisation probe of independent perennial ryegrass plants (lower centre right). Northern hybridisation analysis of perennial ryegrass plants co-transformed with antisense *Lp*Omt1 vector (bottom). C = negative control untransformed perennial ryegrass; P = positive plasmid control.

Figure 36 shows biochemical analysis of *Lp*Omt1-transgenic perennial ryegrass. OMT activity of leaf samples from selected independent *Lp*Omt1-transgenic perennial ryegrass plants (Ell8, Ell11, Ell14 and Ell15) was determined and compared to untransformed perennial ryegrass negative control plant *L. perenne* cv. Ellett (wild type). Mean values and standard deviations of replicate assays are shown.

Figure 37 shows PCR screening of transgenic ryegrass plants. PCR analysis using *npt2*-specific primers of 8 independent transgenic perennial ryegrass plants from biolistic transformation with antisense *Lp*Ubi4CL2 vector.

Figure 38 shows the nucleotide sequence of genomic clone 4CL2 from perennial ryegrass (Sequence ID No: 17).

Figure 39 shows the nucleotide sequence of genomic clone CCR1 from perennial ryegrass (Sequence ID No: 18).

Figure 40 shows the map location of *Lp*4CL1, *Lp*4CL3, *Lp*CAD1, *Lp*CAD2, *Lp*CCR1, *Lp*OMT1 and *Lp*OMT2 (in bold) within the genetic linkage map of perennial ryegrass.

EXAMPLE 1

Isolation and characterisation of three 4-Coumarate CoA-Ligase (4CL) cDNAs from *Lolium perenne*

Materials and Methods

Plant material

Plants and embryogenic cell suspensions of perennial ryegrass (*Lolium perenne* L.) cv Ellet and tall fescue (*Festuca arundinacea* Schreb.) cv Triumph were established and maintained as previously described (Heath et al., 1998). Wounding experiments were performed with 10-day-old seedlings of perennial ryegrass (cv Ellet) as previously described (Heath et al., 1998).

Screening of a cDNA library

A cDNA library prepared with RNA isolated from perennial ryegrass seedlings (Heath et al., 1998) was screened with a [^{32}P]dCTP-labelled rice partial 4CL probe. The rice 4CL probe and consisted of a 844 bp 4CL specific sequence inserted into PUC119. This insert has 93 % sequence identity with a rice 4CL cDNA sequence (Genbank, L43362, bases 453-1300). cDNA inserts were excised and recircularized using the ExAssist helper phage with SOLR strain (Stratagene) as described by the manufacturer.

DNA sequencing

cDNA clones were digested with 8 restriction enzymes (*BamH*I, *EcoR*I, *Kpn*I, *Not*I, *Pst*I, *Sal*I, *Xba*I, *Xho*I) and selected clones were sequenced on both

strands by the dideoxy chain termination method using M13 forward and reverse primers. For sequencing the internal regions of *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 synthetic oligonucleotide primers were designed from the DNA sequences previously determined. Sequencing was performed using the ABI dye terminator kit and automatic sequencer. Nucleotide sequences were aligned using the SeqEd program (ABI) and further analysis was performed using the HIBIO DNASIS vs2 program (Hitachi Software Engineering).

Genomic DNA blot analysis

Genomic DNA was isolated from single genotype-derived cell suspensions of perennial ryegrass and tall fescue according to Lichtenstein and Draper (1985). Ten µg of perennial ryegrass DNA and 20 µg of tall fescue DNA was digested with each of the restriction enzymes *Hind*III and *Xba*I, separated on 1 % agarose gels, and transferred to Hybond N$^+$ membranes according to the manufacturer's instructions (Amersham). Probes consisted of BamHI/KpnI fragments of *Lp*4CL1 (1771 bp), *Lp*4CL2 (2034 bp) or *Lp*4CL3 (2080 bp) labelled using the Megaprime labelling kit (Amersham) and [^{32}P]dCTP. Hybridization was performed at 65 ^{o}C in 5 X SSPE, 5 X Denhardt's solution, 0.5 % (w/v) SDS, and 200 µg/mL denatured herring sperm DNA. Membranes were washed three times in 2 X SSPE, 0.1 % SDS for 10 min at 25 ^{o}C and then twice in 0.1 X SSPE, 0.1 % SDS for 20 min at 65 ^{o}C.

RNA blot analysis

Total RNA (10 µg) was separated on 1.2 % formaldehyde gels and transferred to Hybond N (Amersham) membranes according to the manufacturers instructions. Membranes were stained with 0.2 % methylene blue to confirm correct loading and transfer of RNA. Hybridisation was performed at 42 ^{o}C in 5 X SSPE, 5 X Denhart's solution, 0.5 % SDS, 50 % deionized formamide, 200 µg/mL denatured herring sperm DNA. Preparation of probes and washing of membranes was as for DNA blot analysis except for the tall fescue Northern blot when the final two washes were performed with 0.1 X SSPE, 0.1 % SDS for 10 min at 42°C.

Results

Isolation and sequence analysis of perennial ryegrass 4CL cDNAs

A cDNA library prepared from RNA extracted from perennial ryegrass seedlings was screened with a rice 4CL hybridization probe and ten cDNAs were isolated from 2 X 10^5 pfu. The cDNAs were characterised by restriction analysis with 8 restriction enzymes. All clones were full length (approximately 2.0-2.2 kb) with poly(A) tails and could be separated into three groups: *Lp*4CL1 (four clones) *Lp*4CL2 (five clones) and *Lp*4CL3 (one clone). Plasmid maps for *Lp*4CL1, *Lp*4CL2 and Lp4CL3 are shown (Figure 1). *Lp*4CL1, *Lp*4CL2 and Lp4CL3 were fully sequenced (Figures 2, 3 and 4, respectively).

*Lp*4CL1 is 2284 bp long with an open reading frame (ORF) of 1710 bp, a 5' noncoding region of 322 bp and a 3' noncoding region of 252 bp including a poly(A) tail. *Lp*4CL2 is 1992 bp long with an ORF of 1668 bp, a 5' noncoding region of 61 bp and a 3' noncoding region of 263 bp including a poly(A) tail. *Lp*4CL3 is 2038 bp long with an ORF of 1671 bp, a 5' noncoding region of 112 bp and a 3' noncoding region of 255 bp including a poly(A) tail.

Within the coding region, *Lp*4CL1 has 70 % nucleic acid sequence identity with both *Lp*4CL2 and *Lp*4CL3, while *Lp*4CL2 has 79 % sequence identity with *Lp*4CL3. There is little sequence homology in the 3' noncoding regions between clones (52-55 %).

Amino acid sequence comparisons

The putative proteins encoded by the three cDNAs consist of 570 amino acids [60290 u (Da)] for *Lp*4CL1, 556 amino acids (59238 u) for *Lp*4CL2 and 557 amino acids (59735 u) for *Lp*4CL3. The deduced amino acid sequences of *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 are shown (Figure 5). *Lp*4CL2 and *Lp*4CL3 share 79 % amino acid sequence identity, *Lp*4CL1 and *Lp*4CL2 have 61 % amino acid sequence identity, while *Lp*4CL1 and *Lp*4CL3 have only 58 % amino acid sequence identity. Regions of high sequence homology are more prevalent in the central and c-terminal regions of the enzyme. For example the

sequence identity between amino acids 208 to 568 of each enzyme is 85 % for *Lp*4CL2 and *Lp*4CL3, 72 % for *Lp*4CL1 and *Lp*4CL2 and 67 % for *Lp*4CL1 and *Lp*4CL3.

*Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 share several common regions with other plant 4CLs. In particular, they contain the putative AMP-binding domain and the conserved GEICIRG motif, except for *Lp*4CL3 where the second isoleucine has been replaced with valine (Figure 5). It has been proposed that domain II is associated with the catalytic activity of 4CL. Also, four Cys residues conserved in plant 4CLs are conserved in *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 (Figure 5). These results suggest that the *L. perenne* cDNAs encode three divergent 4CL enzymes that are likely to have originated from three different 4CL genes.

Expression of perennial ryegrass 4CL genes

*Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 were used as hybridization probes in Northern blots with RNA prepared from different organs of perennial ryegrass at two developmental stages. All three probes hybridized to a single mRNA species of approximately 2.2 - 2.3 kb. *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 were expressed at both seedling and mature stages of development and in all organs tested. For *Lp*4CL2 and *Lp*4CL3 the strongest signal was found in RNA samples from seedling roots and mature stems (Figure 6).

*Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 were also used as hybridization probes in Northern blots with RNA prepared from tall fescue. All three probes hybridized to a similar mRNA species (2.3 kb) as that in perennial ryegrass (Figure 6). The strongest signal was found in RNA samples from mature stems with weaker signals in RNA from roots and seedling shoots. No expression of *Lp*4CL1, *Lp*4CL2 or *Lp*4CL3 was observed in leaves. The three probes varied in their ability to hybridize to the corresponding homologues in tall fescue, with *Lp*4CL3 resulting in the highest signal and *Lp*4CL1 hybridizing only weakly.

To determine whether 4CL could be induced under stress conditions,

leaves of perennial ryegrass seedlings were wounded. No increase in the transcript level upon wounding was observed with *Lp*4CL1, *Lp*4CL2 or *Lp*4CL3 (Figure 7).

Genomic organization of perennial ryegrass 4CL genes

Perennial ryegrass DNA was digested with two restriction enzymes, *Hind*III or *Xba*I. Restriction sites for these enzymes are not present in the cDNA sequence of *Lp*4CL1, *Lp*4CL2 or *Lp*4CL3. When *Lp*4CL1, *Lp*4CL2 or *Lp*4CL3 was used as a probe, several DNA hybridizing fragments of varying intensity were revealed (Figure 8). Each probe hybridized to a unique set of fragments, suggesting that *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 represent three different genes. Furthermore, *Lp*4CL1 and *Lp*4CL2 hybridized to 2 to 3 major fragments per digest which may represent either alleles of the same gene or indicate the presence of more than one gene in each class. The *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 probes also revealed several different size hybridizing DNA fragments in genomic Southern blots from tall fescue under high stringency conditions (Figure 8), suggesting that three similar 4CL genes are present in *F. arundinacea*.

EXAMPLE 2

Isolation and characterisation of a Cinnamoyl CoA Reductase (CCR) cDNA from *Lolium perenne*

A total of 500,000 phage were screened from a cDNA library constructed from ten-day-old etiolated *L. perenne* seedlings using a maize CCR probe. Ninety-three positive plaques were observed in the primary screen and five were subsequently analysed by restriction enzyme digestion. Four out of the five were identical. One of the four identical cDNAs, *Lp*CCR1, was selected for further analysis (Figure 9).

Nucleic acid sequence analysis of perennial ryegrass CCR cDNA

The full nucleotide sequence of *Lp*CCR1 was obtained and the amino

acid sequence predicted (Figure 10). *Lp*CCR1 is a 1395 bp cDNA with 149 bp of 5' non-coding region and 160 bp of 3' non-coding region. An open reading frame of 1086 bp encodes a protein of 362 amino acids. The composition of the coding region was found to be 68% G+C rich. Codon usage was also examined and found to be biased towards XXC/G codons (94%), with XCG and XUA codons accounting for only 9% and 0.55% respectively. G+C richness and bias towards G and C in the third position of a codon triplet are previously reported characteristics of monocot genes.

Genomic organization of perennial ryegrass CCR gene

The number of CCR genes present in the ryegrass genome was determined by Southern blot analysis of genomic DNA from double haploid plants, using as probe a fragment of the *Lp*CCR1 cDNA (LpCCR531, Figure 9). Double haploid DNA reduces the complexity associated with allelic variation. Genomic DNA was cut with enzymes that do not cut the cDNA internally; *Dra*I, *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I, and the membrane was hybridised and washed under medium-stringency conditions. A single strongly hybridising band was evident in each lane (Figure 11) indicating that there is a single copy of the *Lp*CCR1 gene in the perennial ryegrass genome.

Expression of perennial ryegrass CCR gene

To investigate the expression profile of the CCR gene in ryegrass, northern hybridisation analysis was carried out with total RNA extracted from roots and shoots at seedling growth stages (0.5-1cm and 4-6cm shoots) and roots, stem and leaves at mature growth stages (6 and 10 weeks). Seedlings were grown on filter paper in the dark at 25°C and then transferred to soil and glasshouse conditions (25°C) until the 6 and 10-week stages. Whole seedling total RNA from *Festuca* and *Phalaris* was included in the northern analysis. Hybridisation with *Lp*CCR531 (Figure 9) was performed at medium-stringency and the membrane was then washed at high-stringency. A transcript of approximately 1.5 kb was detected in all tissues, the level of expression varying with maturity and from one tissue type to another (Figure 12). The *Lp*CCR1 transcript appears to be more abundant in roots and stem than

shoots and leaves. In the stem, transcript abundance increases from 6-weeks to 10-weeks; indicating that transcription in stem tissue is up-regulated as the plant matures. Expression was found predominantly in tissues such as stems and roots that are forming secondary cell walls indicating that *Lp*CCR1 is constitutively involved in lignification.

EXAMPLE 3

Isolation and characterisation of Cinnamyl Alcohol Dehydrogenase (CAD) cDNAs from *Lolium perenne*

A 558 bp cinnamyl alcohol dehydrogenase (CAD) fragment was amplified from cDNA synthesised from total RNA prepared from perennial ryegrass seedlings. The conserved amino acid domains between *Pinus radiata, Medicago sativa, Aralia cordata, Eucalyptus botryoides* and *Arabidopsis thaliana* CADs were used to design oligonucleotides for the amplification of the perennial ryegrass CAD. The forward oligonucleotide was designed to the conserved amino acid domain CAGVTVYS and the reverse oligonucleotide to the conserved domain DVRYRFV. The 551 bp PCR fragment was cloned and sequenced to confirm that it corresponded to a perennial ryegrass CAD PCR fragment. A cDNA library prepared from RNA extracted from perennial ryegrass seedlings was screened with the 551bp PCR fragment specific for perennial ryegrass CAD. Eight cDNAs were isolated and separated into six groups by restriction digest analysis. One representative clone each from two groups (*Lp*CAD1, *Lp*CAD2) were selected for further characterisation.

Nucleic acid sequence analysis of perennial ryegrass CAD cDNAs

The complete sequence of the perennial ryegrass CAD homologue *Lp*CAD1 was determined (Figure 13). The 1325 bp clone had a poly (A) tail, typical start and stop codons and the open reading frame (ORF) of this clone coded for a putative protein of 408 amino acids.

The complete nucleotide sequence of the perennial ryegrass CAD homologue *Lp*CAD2 was also determined (Figure 14).

Expression of perennial ryegrass CAD genes

A northern hybridisation analysis with RNA samples isolated from perennial ryegrass at different developmental stages hybridised with the full length *Lp*CAD1 1325 bp cDNA (Figure 15) was performed to determine patterns of organ and developmental expression. The probe hybridised to a single mRNA species of approximately 1.6 kb. The *Lp*CAD1 transcript was expressed in all tissue tested: roots, shoots, stem and leaves (Figure 16A). The *Lp*CAD1 transcript was most abundant in root tissue and the mature stem, this expression pattern is typical of a gene involved in the lignification of plant cell walls. Intergeneric homologies were revealed in *Festuca* and *Phalaris*.

A similar northern hybridisation analysis was performed with *Lp*CAD2 (Figure 16B), however the transcript was found to be most abundant in mature stem tissue and the shoots.

Genomic organization of perennial ryegrass CAD genes

A Southern hybridisation analysis using DNA samples isolated from a perennial ryegrass double haploid plant digested with *Dra*I, *Bam*HI, *Eco*RI, *Eco*RV, *Hind*III and *Xba*I and hybridised with a 500 bp *Lp*CAD1 probe was performed. The hybridisation pattern at high stringency revealed the presence of two prominent bands for most digests indicating that *Lp*CAD1 belongs to a small gene family and exists a muliticopy gene in perennial ryegrass (Figure 17A).

A similar Southern hybridization analysis was performed with *Lp*CAD2 (Figure 17B) the hybridisation pattern at high stringency revealed the presence of one or two prominent bands for most digests indicating that *Lp*CAD2 exists as a single copy gene or a member of a small gene family in perennial ryegrass (Figure 17B).

EXAMPLE 4

Isolation and characterisation of genomic clones and promoters for *O*-methyltransferase (OMT), cinnamoyl-CoA reductase (CCR), 4 coumarate CoA-ligase (4CL) and cinnamyl alcohol dehydrogenase (CAD) from *Lolium perenne*

Genomic clones and promoters of *O*-methyltransferase (OMT), cinnamoyl-CoA reductase (CCR), 4 coumarate CoA-ligase (4CL) and cinnamyl alcohol dehydrogenase (CAD) were isolated from a perennial ryegrass genomic library using the corresponding cDNAs as hybridisation probes.

Isolation and characterisation of genomic clones and promoters for perennial ryegrass O-methyltransferase (OMT)

A perennial ryegrass genomic library was screened with the cDNA clone, *Lp*Omt1, (Heath *et al.* 1998) encoding *O*-methyltransferase (OMT). The sequence of the 5' untranslated region and the coding region was found to be identical to that of the *Lp*Omt1 cDNA previously isolated. The entire 4.8 kb genomic clone was fully sequenced (Figure 18).

To further characterise the promoters, transcriptional fusions of the promoter sequence to the β-glucuronidase (GUS) coding sequence (*gusA*) have been generated (Figure 19). Direct gene transfer experiments to tobacco protoplasts were performed with the corresponding chimeric genes to transgenically express them in a heterologous system for *in planta* expression pattern analysis by histochemical GUS assays. A set of transgenic tobacco plants carrying a chimeric *gusA* gene under the control of the 5' regulatory region of the *Lp*Omt1 promoter was generated to assess the potential use of the *Lp*Omt1 promoter for xylem-specificity and targeted downregulation of genes encoding key lignin biosynthetic enzymes.

The transgenic tobacco plants generated using the *Lp*Omt1 promoter driven chimeric *gusA* transformation vector were screened by PCR and histochemical GUS assays.

A PCR screening was undertaken using *gusA* specific primers for the initial identification of transgenic tobacco plants (Figure 20). PCR positive tobacco plants were screened by histochemical GUS assays for *in planta* expression pattern analysis (Figure 20).

Isolation and characterisation of genomic clones and promoters for perennial ryegrass cinnamoyl-CoA reductase (CCR)

A CCR genomic clone from perennial ryegrass was isolated containing 6.5 kb of promoter and the entire gene organisation (intron/exon boundaries). The CCR promoter can be used for targeted expression of foreign genes in transgenic plants.

A perennial ryegrass genomic library was screened with the cDNA clone *Lp*CCR1 which codes for the lignin biosynthetic enzyme, cinnamyl-CoA reductase (CCR). Four different genomic clones were identified based on restriction digest analysis. Clone 6.1.1a was selected for further analysis. A 6.42 kb *Xho*I fragment from clone 6.1.1a, which hybridized strongly to the *Lp*CCR1 cDNA probe, was subcloned into pBluescriptSK (Figure 21A). Sequence analysis revealed that the 6.42 kb *Xho*I fragment contained the entire *Lp*CCR1 gene and 200 bp of promoter region. The intron/exon boundaries are illustrated in figure 21B, the location and the size of the exons appear to be conserved in other CCRs from different species (Figure 21C).

To isolate the promoter region of *Lp*CCR1, the Southern blot containing digested phage genomic DNA isolated from clone λLp6.1.1a was reprobed with the 200bp promoter region. The probe hybridized strongly to a 6.5 kb *Sal*I fragment. This genomic fragment *Lp*CCR1 clone 2, was subcloned into pBluescriptSK and sequenced (Figure 22A). Sequence results revealed that the 6.5 kb *Sal*I fragment contained 6.5 kb of promoter (Figure 22B). The full sequence of *Lp*CCR1 genomic clone containing the promoter and entire gene sequence (exons and introns) was obtained and is shown on Figure 39.

Isolation and characterisation of genomic clones and promoters for perennial ryegrass 4 coumarate CoA-ligase (4CL)

A 4CL2 genomic clone from perennial ryegrass was isolated containing 2.5 kb of promoter and partial gene organisation (intron/exon boundaries). The 4CL2 promoter can be used for targeted expression of foreign genes in transgenic plants. The 2.5 kb promoter has been fused to the reporter gene *gusA* for expression analysis.

A perennial ryegrass genomic library was screened with an *Lp*4CL cDNA probe. After tertiary screening positive 4CL genomic clones were obtained and characterised by restriction digest and Southern hybridisation analysis (Figure 23A).

Sequence analysis revealed that the isolated 4CL genomic clone (4CL2) from perennial ryegrass had 100% nucleotide identity to the *Lp*4CL2 cDNA clone. To further characterise this 5 kb λ*Lp*4CL2 genomic clone and to confirm that it corresponds to the cDNA of *Lp*4CL2, a number of PCR reactions using primers designed to the cDNA were used. PCR results confirmed that the 5 kb genomic fragment was a partial genomic clone corresponding to the *Lp*4CL2 cDNA (Figure 23B). Using primer combinations F1 and R1 the entire 4.8kb genomic fragment was amplified. To determine the location of introns additional PCR reactions using the primer combinations F1 / R2 and F2 / R1 were performed, a 1 kb and 3.5 kb bands were amplified respectively. The location and size of the introns could be determined from these results, and further confirmed by sequence analysis. This large 5 kb genomic fragment contains 4 small exons representing the coding sequence of *Lp*4CL2 between 508 bp and 1490 bp (Figure 23C).

The genomic clone 1, *Lp*4CL2 contained no promoter region. To isolate the promoter region of *Lp*4CL2, the Southern blot containing digested phage genomic DNA isolated from clone λ*Lp*4CL2 was reprobed with a 300 bp EcoRI/BglI isolated from the 5' end of the cDNA clone *Lp*4CL2. The 300 bp probe hybridised strongly to a 2.5 kb BamHI fragment. This genomic fragment

*Lp*4CL2 clone 2, was subcloned into pBluescriptSK and sequenced (Figure 24A). Sequence results revealed that the 2.5 kb *Bam*HI fragment contained the 508 bp of the 5' ORF of *Lp*4CL2 missing from genomic clone 1 and 2.0 kb of promoter region (Figure 24B). The full sequence of the *Lp*4CL2 genomic clone containing the promoter and partial gene sequence (exons and introns) was obtained and is shown on Figure 39.

The promoter from *Lp*4CL2 was thus isolated and used for the production of a chimeric *gusA* reporter gene (Figure 25).

Isolation and characterisation of genomic clones and promoters for perennial ryegrass cinnamyl alcohol dehydrogenase (CAD)

A CAD genomic clone from perennial ryegrass was isolated containing the gene organisation (intron/exon boundaries) minus intron 1 containing the first 111 bp of the CAD coding region. The genomic clone has allowed the identification of a G at position 851 bp in the coding region of the CAD2 genomic clone isolated from perennial ryegrass cv. Barlano which is absent in the CAD2 cDNA clone isolated from perennial ryegrass cv. Ellett. The SNP (single nucleotide polymorphism) found to exist between the 2 cultivars has the potential utility as a molecular marker for herbage quality, dry matter digestibility, mechanical stress tolerance, disease resistance, insect pest resistance, plant stature and leaf and stem colour.

Results below show the isolation of the genomic clone and sequence analysis of deduced coding sequence from the genomic clone CAD2 from perennial ryegrass cv. Barlano compared to the truncated cDNA CAD2 from the cv Ellett. The missing G in the perennial ryegrass cv. Ellett has been highlighted (Figures 26 and 27).

A perennial ryegrass genomic library was screened with a probe corresponding to the 5' end of the *Lp*CAD2 cDNA clone, which codes for the lignin biosynthetic enzyme cinnamyl alcohol dehydrogenase. Ten positive plaques were identified and isolated in the primary library screening. After a secondary and tertiary screening, two positive plaques were obtained and

corresponding positive genomic clones were further characterised by restriction digest and Southern hybridization analyses. Both genomic clones were found to be identical based on restriction digest analyses. One clone, named λ*Lp*CAD2 was chosen for further Southern hybridization analyses. A 4.5 kb *Bam*HI fragment which hybridized strongly to the *Lp*CAD2 cDNA probe was subcloned into pBluescriptSK and sequenced (Figure 28A). Sequence analysis revealed that the 4.5 kb *Bam*HI fragment was a partial genomic clone of *Lp*CAD2. This large 4.5 kb genomic fragment contains 4 small exons representing the coding sequence of *Lp*CAD2 between 213 bp and the stop codon at 1213 bp, and the location of the intron/exon boundaries are illustrated in Figure 28B.

EXAMPLE 5

Development of transformation vectors containing chimeric genes with 4CL, CCR and CAD cDNA sequences from perennial ryegrass

To alter the expression of the key enzymes involved in lignin biosynthesis 4CL, CCR and CAD, through antisense and/or sense suppression technology and for over-expression of these key enzymes in transgenic plants, a set of sense and antisense transformation vectors was produced. Transformation vectors containing chimeric genes using perennial ryegrass 4CL, CCR and CAD cDNAs in sense and antisense orientations under the control of either the CaMV 35S or the maize ubiquitin promoter were generated (Figures 29, 30 and 31).

EXAMPLE 6

Production and characterisation of transgenic tobacco plants expressing chimeric 4CL, CCR and CAD genes from perennial ryegrass

A set of transgenic tobacco plants carrying chimeric 4CL, CCR and CAD genes from perennial ryegrass were produced and analysed.

Transformation vectors with *Lp*4CL1, *Lp*4CL2 and *Lp*4CL3 full length cDNA sequences in sense and antisense orientations under the control of either the CaMV 35S or the maize ubiquitin promoters were generated. Transformation vectors with *Lp*CCR1 cDNA in both sense and antisense orientation under the control of either the CaMV 35S and maize ubiquitin promoters were generated. Transformation vectors with 1325 bp full length *Lp*CAD1 cDNA in sense and 1051 bp partial *Lp*CAD1 cDNA in antisense orientation under the control of either the CaMV 35S and maize ubiquitin promoters were generated.

Direct gene transfer experiments to tobacco protoplasts were performed using these transformation vectors.

The production and molecular analysis of transgenic tobacco plants carrying the perennial ryegrass *Lp*4CL1 and *Lp*CCR1 cDNAs under the control of the constitutive CaMV 35S promoter is described here in detail.

A set of transgenic tobacco plants generated using the *Lp*4CL1 sense transformation vector was screened by PCR and subjected to Southern and northern hybridization analyses.

A PCR screening was undertaken using *npt2* and *Lp*4CL1 specific primers for the initial identification of transgenic tobacco plants. Independent transgenic tobacco plants were identified to be co-transformed with both the selectable marker *npt2* and the *Lp*4CL1 chimeric genes (Figure 32).

Southern hybridisation analysis was performed with DNA samples from PCR positive transgenic tobacco plants to demonstrate the integration of the chimeric *Lp*4CL1 transgene in the tobacco plant genome. Independent transgenic tobacco plants carried between 1 and 5 copies of the *Lp*4CL1 transgene. No cross-hybridization was observed between the endogenous tobacco 4CL gene and the perennial ryegrass hybridization probe used (Figure

32).

Northern hybridization analysis using total RNA samples prepared from the transgenic tobacco plants carrying the chimeric sense *Lp*4CL1 transgene and probed with the *Lp*4CL1-specific hybridization probe revealed the presence of a 1.2 kb *Lp*4CL1 transcript strongly expressed in one *Lp*4CL1-transgenic tobacco plant analysed (Figure 32).

The sense and antisense transformation vectors of *Lp*CCR1 under the control of the CaMV 35S promoter were introduced into tobacco protoplasts via direct gene transfer. A set of transgenic tobacco plants was generated and screened by PCR with specific primers to identify transgenic tobacco plants carrying chimeric *Lp*CCR1 transgene. The molecular analysis of *Lp*CCR1-transgenic tobacco plants is shown (Figure 33).

EXAMPLE 7

Production and characterisation of transgenic perennial ryegrass plants expressing chimeric OMT, 4CL, CCR and CAD genes from perennial ryegrass

An improved transformation method was developed for the production of transgenic perennial ryegrass plants by biolistic transformation of embryogenic cells. Transgenic perennial ryegrass plants were generated using chimeric OMT, 4CL, CCR and CAD genes from perennial ryegrass and the improved transformation method.

Improved method for the production of transgenic perennial ryegrass plants

This improved procedure utilises embryogenic calli produced from mature seed-derived embryos as direct targets for biolistic transformation without requiring the establishment of embryogenic cell suspensions. The protocol relies on a continuous supply of isolated zygotic embryos for callus induction. Transgenic ryegrass plants can be regenerated 24 – 28 weeks after

embryo isolation (Fig. 34). Isolated embryos are plated onto MSM5 medium to produce embryogenic calli suitable as targets for biolistic transformation within 8 weeks. The embryogenic calli, treated on high-osmoticum medium MSM3 Plus prior to microprojectile bombardment, are selected on MSM3 medium containing 100 mg/l paromomycin (Pm) for 2 weeks before being transferred onto MSK with 100 mg/l Pm for further 4 weeks until differentiation of Pm resistant shoot appear. Regenerated shoots are transferred on to fresh selective media MSK with 100 mg/l Pm for a further 4 weeks (Figure 34).

Production of transgenic perennial ryegrass plants expressing chimeric OMT, 4CL, CCR and CAD genes from perennial ryegrass

Transgenic perennial ryegrass (*Lolium perenne*) plants were generated using chimeric ryegrass OMT, 4CL, CCR and CAD genes by biolistic transformation of embryogenic calli. Examples of the production and detailed molecular analysis of these transgenic ryegrass plants are described.

Transgenic perennial ryegrass plants for OMT down-regulation were produced using biolistic transformation of embryogenic calli and plant transformation vectors pUbiomt1 and pUbitmo1 carrying *Lp*Omt1 cDNA sequence in sense and antisense orientation under control of the constitutive maize ubiquitin promoter. These transgenic perennial ryegrass plants for down-regulated OMT activity were regenerated from paromomycin resistant calli obtained from biolistic transformation using microprojectilies coated with two plasmids; pHP23 (carrying the chimeric *npt2* gene as the selectable marker) and either the sense or antisense *Lp*Omt1 transformation vector driven by the maize *Ubi* promoter.

Transgenic perennial ryegrass plants were subjected to a polymerase chain reaction (PCR) screening using *npt2*-specific primers. Independent *npt2* PCR-positive transgenic perennial ryegrass plants obtained from biolistic transformation of embryogenic calli – generated from approximately 60,000 isolated mature seed-derived embryos - using *Lp*Omt1 sense (pUbiomt1) and *Lp*Omt1 antisense (pUbitmo1) transformation vectors were identified [16 pUbiomt1 transgenic plants and 27 pUbitmo1 transgenic plants] (Figure 35).

Southern hybridization analysis was performed with undigested and *Hind*III-digested DNA samples prepared from the PCR positive transgenic perennial ryegrass plants, to demonstrate their transgenic nature and the integration of the chimeric *npt2* and *Lp*Omt1 transgenes. Independent transgenic perennial ryegrass plants co-transformed with both, the selectable marker *npt2* gene and *Lp*Omt1 chimeric genes, were identified (Figure 35). In most instances, the transgenic perennial ryegrass plants recovered contained multiple copies of the selectable marker gene including rearranged transgene copies. No *npt2*-hybridizing bands were detected in the untransformed negative control.

Samples of *Hind*III-digested genomic DNA were included in the analysis when the *Lp*Omt1 gene-specific hybridization probe (*omt1*) was used. The *omt1* probe hybridized to a number of bands in DNA samples corresponding to both, the transgenic plants and the untransformed negative control. The *omt1*-hybridizing bands shared in all samples correspond to endogenous *Lp*Omt1 gene sequences represented as a small multigene family in the perennial ryegrass genome (Heath et al. 1998). The different *omt1*-hybridizing bands evident in the samples from the transgenic plants and absent in the untransformed negative control sample correspond to antisense (*tmo1*) and sense (*omt1*) *Lp*Omt1 transgene integration events (Figure 35).

Northern hybridization analysis using strand-specific *Lp*Omt1 probes allowed the identification of transgenic perennial ryegrass plants expressing the antisense *Lp*Omt1 transgene (Figure 35).

The OMT activity of selected antisense and sense *Lp*Omt1 transgenic perennial ryegrass plants was determined. Biochemical assays for OMT activity were initially established in untransformed plants (such as tobacco and perennial ryegrass). The assays utilise radiolabelled S-adenosylmethionine as the methyl donor for the OMT-catalysed conversion of caffeic acid into ferulic acid. The production of radioactive ferulic acid is measured and allows the OMT activity to be determined.

The OMT activity of selected *Lp*Omt1-transgenic perennial ryegrass plants (*L. perenne* cv. Ellett) was determined. Significantly altered OMT activity in individual transformation events was observed (Figure 36). The manipulation of OMT activity in transgenic perennial ryegrass plants due to the expression of the chimeric ryegrass *Lp*Omt1 gene was thus demonstrated.

Transgenic perennial ryegrass plants were recovered, using biolistic transformation of embryogenic calli, for the manipulation of the expression of genes encoding the key lignin biosynthetic enzyme, 4CL. The plant transformation vectors pUbi4CL2 and pUbi2LC4 carrying chimeric *Lp*4CL2 cDNA sequences in sense and antisense orientation, respectively, driven by the constitutive maize ubiquitin (*Ubi*) promoter were used. Perennial ryegrass plants for 4CL manipulation were regenerated from Pm-resistant calli obtained from biolistic transformation of embryogenic calli using microprojectiles coated with the plasmids pHP23, carrying a chimeric *npt2* gene as selectable marker gene and the antisense pUbi2LC4.

Transgenic perennial ryegrass plants were subjected to a polymerase chain reaction (PCR) screening using *npt2*-specific primers. Independent *npt2* PCR-positive transgenic perennial ryegrass plants were obtained from biolistic transformation of embryogenic calli (Figure 37).

Transgenic perennial ryegrass plants were also recovered, using biolistic transformation of embryogenic calli, for the manipulation of the expression of genes encoding the key lignin biosynthetic enzymes, CCR and CAD.

EXAMPLE 8

Genetic mapping of perennial ryegrass OMT, 4CL, CCR and CAD genes

*Lp*4CL1, *Lp*4CL3, *Lp*CAD1, *Lp*CAD2, *Lp*CCR1, *Lp*OMT1 and *Lp*OMT2 clones were PCR amplified and radio-labelled for use as probes to detect

restriction fragment length polymorphisms (RFLPs). RFLPs were mapped using 110 progeny individuals of the p150/112 perennial ryegrass reference population restricted with the enzymes described in the table below.

Clones	Polymorphic in p150/112	Enzyme mapped with	Locus	Linkage group
*Lp*4CL1	Y	*Dra*I	*Lp*4CL1	2
*Lp*4CL3	Y	*Eco*RV	*Lp*4CL3	6
*Lp*CAD1	Y	*Eco*RV	*Lp*CAD1	2
*Lp*CAD1.2.1	Y	*Eco*RI	*Lp*CAD2a *Lp*CAD2b *Lp*CAD2c	7 - 2
*Lp*CCR1	Y	*Eco*RI	*Lp*CCR1	7
*Lp*OMT1	Y	*Dra*I	*Lp*OMT1	7
*Lp*OMT2	Y	*Eco*RV	*Lp*OMT2	6

*Lp*4CL1, *Lp*4CL3, *Lp*CAD1, *Lp*CAD2, *Lp*CCR1, *Lp*OMT1 and *Lp*OMT2 loci mapped to the linkage groups as indicated in the table and in Figure 40. These gene locations can now be used as candidate genes for quantitative trait loci for lignin biosynthesis associated traits such as herbage quality, dry matter digestibility, mechanical stress tolerance, disease resistance, insect pest resistance, plant stature and leaf and stem colour.

REFERENCES

Heath *et al* (1988) cDNA cloning and differential expression of three caffeic acid O-methyltransferase homologues from perennial ryegrass (*Lolium perenne*). Journal of Plant Physiology 153:649-657

Lichtenstein, C, And J. Draper (1985) Genetic engineering of plants. *In*: D.M. Glover (ed.), DNA Cloning, Vol. 2, pp. 67-119, IRL Press, Washington.

Finally, it is to be understood that various alterations, modifications and/or additions may be made without departing from the spirit of the present invention as outlined herein.

It will also be understood that the term "comprises" (or its grammatical variants) as used in this specification is equivalent to the term "includes" and should not be taken as excluding the presence of other elements or features.

Documents cited in this specification are for reference purposes only and their inclusion is not an acknowledgement that they form part of the common general knowledge in the relevant art.

CLAIMS

1. A substantially purified or isolated nucleic acid or nucleic acid fragment encoding an enzyme selected from the group consisting of 4 coumarate CoA-ligase (4CL), cinnamoyl-CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD), from a ryegrass (*Lolium*) or fescue *(Festuca)* species.

2. A nucleic acid or nucleic acid fragment according to claim 1 wherein said ryegrass or fescue species is perennial ryegrass *(Lolium perenne)*.

3. A nucleic acid or nucleic acid fragment according to claim 1 encoding 4CL and including a nucleotide sequence selected from the group consisting of (a) sequences shown in Figures 2, 3 and 4 hereto (Sequence ID Nos: 1, 3 and 5; respectively) (b) complements of the sequences shown in Figures 2, 3 and 4 hereto (Sequence ID Nos: 1, 3 and 5, respectively); (c) sequences antisense to the sequences recited in (a) and (b); and (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c).

4. A nucleic acid or nucleic acid fragment according to claim 1 encoding CCR and including a nucleotide sequence selected from the group consisting of (a) the sequence shown in Figure 10 hereto (Sequence ID No: 7); (b) the complement of the sequence shown in Figure 10 hereto (Sequence ID No: 7); (c) sequences antisense to the sequences recited in (a) and (b); and (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c).

5. A nucleic acid or nucleic acid fragment according to claim 1 encoding CAD and including a nucleotide sequence selected from the group consisting of (a) the sequences shown in Figures 13, 14, 26 and 27 hereto (Sequence ID Nos: 9, 11, 14 and 16, respectively); (b) complements of the sequences shown in Figures 13, 14, 26 and 27 hereto (Sequence ID Nos: 9,

11, 14 and 16, respectively); (c) sequences antisense to the sequences recited in (a) and (b); and (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c).

6. A vector including a nucleic acid or nucleic acid fragment according to claim 1.

7. A vector according to claim 6 further including a promoter and a terminator, said promoter, nucleic acid or nucleic acid fragment and terminator being operatively linked.

8. A plant cell, plant, plant seed or other plant part, including a vector according to claim 6.

9. A method of modifying lignin biosynthesis in a plant, said method including introducing into said plant an effective amount of a nucleic acid or nucleic acid fragment according to claim 1 and/or a vector according to claim 6.

10. Use of a nucleic acid or nucleic acid fragment according to claim 1, and/or nucleotide sequence information thereof, and/or single nucleotide polymorphisms thereof as a molecular genetic marker.

11. A substantially purified or isolated polypeptide from a ryegrass *(Lolium)* or fescue *(Festuca)* species, selected from the group consisting of the enzymes 4CL, CCR and CAD.

12. A polypeptide according to claim 11 wherein said ryegrass or fescue species is perennial ryegrass *(Lolium perenne)*.

13. A polypeptide according to claim 11 wherein said polypeptide is 4CL and includes an amino acid sequence selected from the group consisting of sequences shown in Figures 2, 3 and 4 hereto (Sequence ID Nos: 2, 4 and 6, respectively); and functionally active fragments and variants thereof.

14. A polypeptide according to claim 11 wherein said polypeptide is CCR and includes an amino acid sequence selected from the group consisting of the sequence shown in Figure 10 hereto (Sequence ID No: 8); and functionally active fragments and variants thereof.

15. A polypeptide according to claim 11 wherein said polypeptide is CAD and includes an amino acid sequence selected from the group consisting of the sequence shown in Figures 13, 14, 26 and 27 hereto (Sequence ID Nos: 10, 12, 15 and 17, respectively); and functionally active fragments and variants thereof.

16. A lignin or modified lignin substantially or partially purified or isolated from a plant, plant seed or other plant part according to claim 8.

17. An isolated regulatory element capable of causing expression of an exogenous gene in plant cells, wherein said regulatory element is isolated from a nucleic acid or nucleic acid fragment encoding a protein selected from the group consisting of: O-methyl transferase (OMT), 4 coumarate CoA-ligase (4CL), cinnamoyl-CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD).

18. A regulatory element according to claim 17 wherein said regulatory element includes an *O*-methyltransferase promoter.

19. A regulatory element according to claim 17 wherein said regulatory element includes a 4 coumarate CoA-ligase promoter.

20. A regulatory element according to claim 17 wherein said regulatory element includes a cinnamoyl CoA-reductase promoter.

19. A regulatory element according to claim 17 from a ryegrass *(Lolium)* or Fescue (*Festuca)* species.

20. A regulatory element according to claim 17 including the first

approximately 4630 nucleotides of the sequence shown in Figure 18 hereto (Sequence ID No: 13); or a functionally active fragment or variant thereof.

21. A regulatory element according to claim 17 including the first approximately 2206 nucleotides of the sequence shown in Figure 38 hereto (Sequence ID No: 17); or a functionally active fragment or variant thereof.

22. A regulatory element according to claim 17 including the first approximately 6735 nucleotides of the sequence shown in Figure 39 hereto (Sequence ID No: 18); or a functionally active fragment or variant thereof.

23. A vector including a regulatory element according to claim 17.

24. A vector according to claim 23 further including an exogenous gene and a terminator, said regulatory element, exogenous gene and terminator being operatively linked, such that said regulatory element is capable of causing expression of said exogenous gene in plant cells.

25. A plant cell, plant, plant seed or other plant part, including a vector according to claim 23.

26. A method for expressing an exogenous gene in plant cells, said method including introducing into said plant cells an effective amount of a regulatory element according to claim 17 and/or a vector according to claim 23.

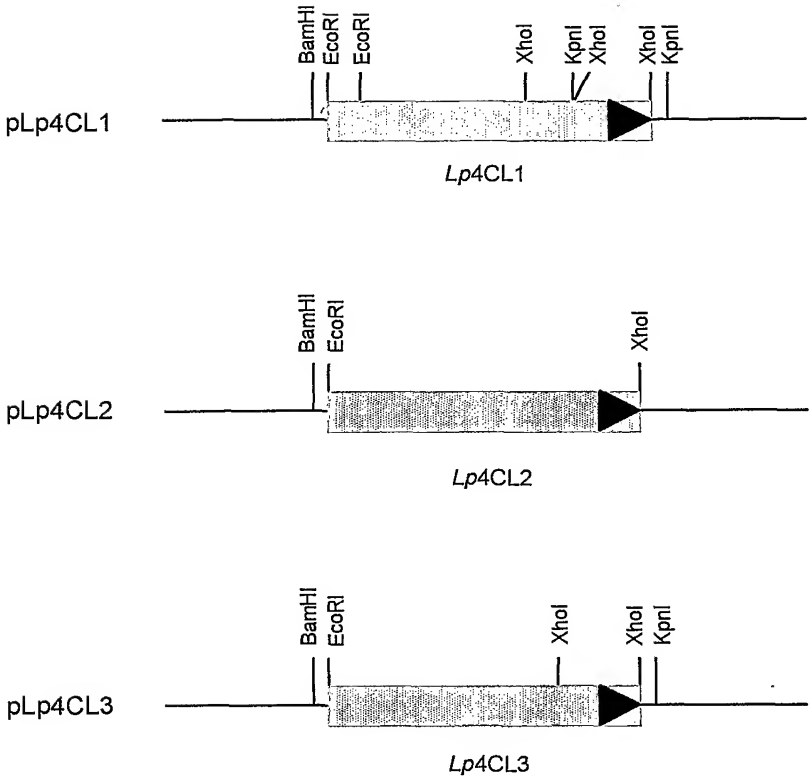
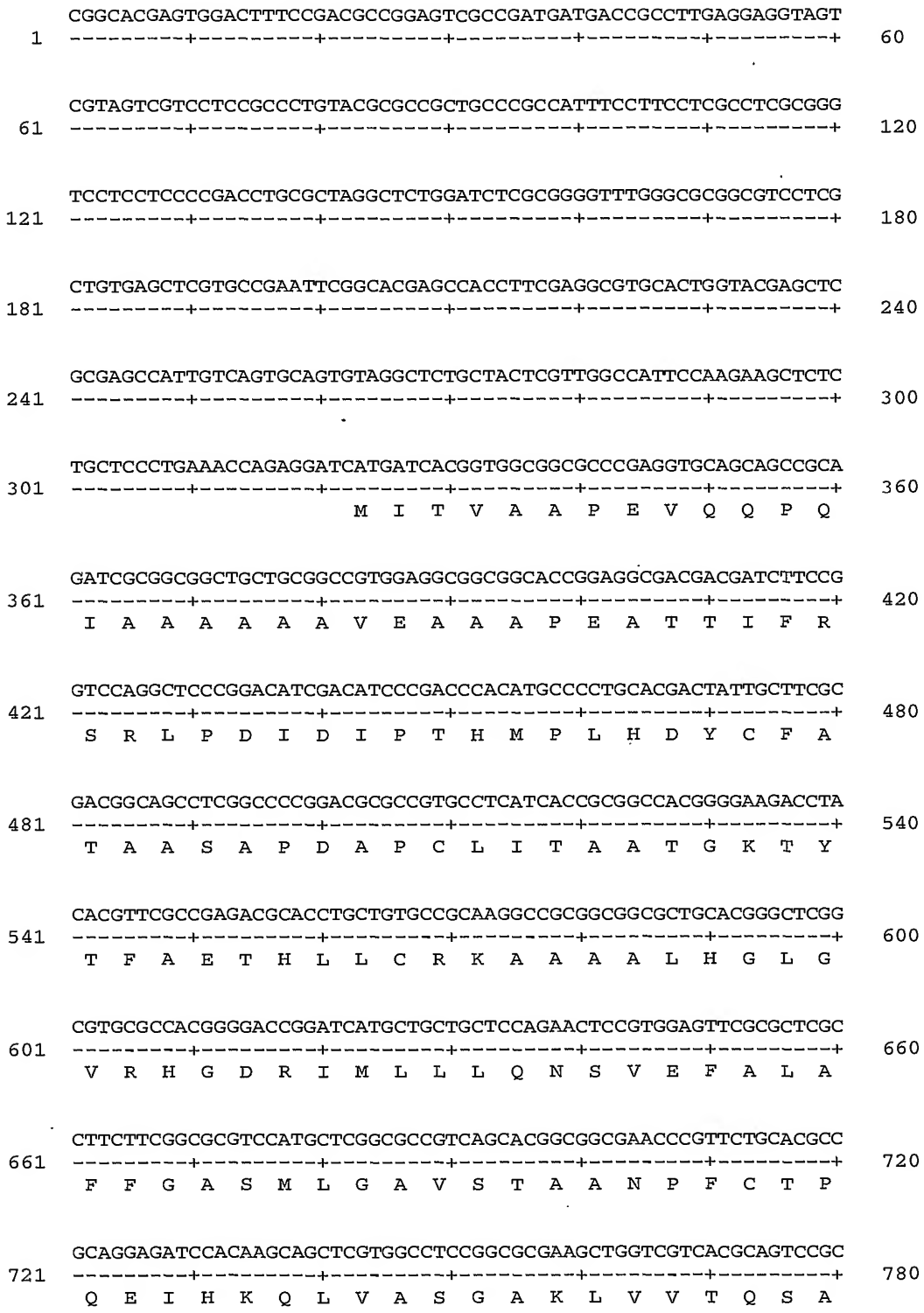

BamHI
EcoRI
EcoRI
XhoI
KpnI
XhoI
XhoI
KpnI
pLp4CL1
Lp4CL1
BamHI
EcoRI
XhoI
pLp4CL2
Lp4CL2
BamHI
EcoRI
XhoI
XhoI
KpnI
pLp4CL3
Lp4CL3

FIGURE 1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1 60
CGTAGTCGTCCTCCGCCCTGTACGCGCCGCTGCCCGCCATTTCCTTCCTCGCCTCGCGGG
61 120
TCCTCCTCCCCGACCTGCGCTAGGCTCTGGATCTCGCGGGGTTTGGGCGCGGCGTCCTCG
121 180
CTGTGAGCTCGTGCCGAATTCGGCACGAGCCACCTTCGAGGCGTGCACTGGTACGAGCTC
181 240
GCGAGCCATTGTCAGTGCAGTGTAGGCTCTGCTACTCGTTGGCCATTCCAAGAAGCTCTC
241 300
TGCTCCCTGAAACCAGAGGATCATGATCACGGTGGCGGCGCCCGAGGTGCAGCAGCCGCA
301 360
M I T V A A P E V Q Q P Q
GATCGCGGCGGCTGCTGCGGCCGTGGAGGCGGCGGCACCGGAGGCGACGACGATCTTCCG
361 420
I A A A A A A V E A A A P E A T T I F R
GTCCAGGCTCCCGGACATCGACATCCCGACCCACATGCCCCTGCACGACTATTGCTTCGC
421 480
S R L P D I D I P T H M P L H D Y C F A
GACGGCAGCCTCGGCCCCGGACGCGCCGTGCCTCATCACCGCGGCCACGGGGAAGACCTA
481 540
T A A S A P D A P C L I T A A T G K T Y
CACGTTCGCCGAGACGCACCTGCTGTGCCGCAAGGCCGCGGCGGCGCTGCACGGGCTCGG
541 600
T F A E T H L L C R K A A A A L H G L G
CGTGCGCCACGGGGACCGGATCATGCTGCTGCTCCAGAACTCCGTGGAGTTCGCGCTCGC
601 660
V R H G D R I M L L L Q N S V E F A L A
CTTCTTCGGCGCGTCCATGCTCGGCGCCGTCAGCACGGCGGCGAACCCGTTCTGCACGCC
661 720
F F G A S M L G A V S T A A N P F C T P
GCAGGAGATCCACAAGCAGCTCGTGGCCTCCGGCGCGAAGCTGGTCGTCACGCAGTCCGC
721 780
Q E I H K Q L V A S G A K L V V T Q S A

FIGURE 2

```
                CTACGTCGACAAGCTCCGGCACGAGGCCTTCCCCCGAATCGGCGAGGCCCTCACCGTGAT
781             ---------+---------+---------+---------+---------+---------+  840
                 Y  V  D  K  L  R  H  E  A  F  P  R  I  G  E  A  L  T  V  I

                CACCATCGACGAGGACGACGGCACCCCGGACGGCTGCCAGCCGTTCTGGGCCCTCGTGTC
841             ---------+---------+---------+---------+---------+---------+  900
                 T  I  D  E  D  D  G  T  P  D  G  C  Q  P  F  W  A  L  V  S

                AGCCGCCGACGAGAACAGCGTCCCGGAGTCTCCCATCTCGCCGGACGACGCGGTGGCGCT
901             ---------+---------+---------+---------+---------+---------+  960
                 A  A  D  E  N  S  V  P  E  S  P  I  S  P  D  D  A  V  A  L

                GCCCTACTCGTCGGGCACGACGGGGCTGCCCAAGGGCGTGGTGCTGACGCACGGGGGGCT
961             ---------+---------+---------+---------+---------+---------+ 1020
                 P  Y  S  S  G  T  T  G  L  P  K  G  V  V  L  T  H  G  G  L

                GGTGTCGAGCGTGGCGCAGCAGGTGGACGGCGAGAACCCGAACCTGCACATGCGGGCGGG
1021            ---------+---------+---------+---------+---------+---------+ 1080
                 V  S  S  V  A  Q  Q  V  D  G  E  N  P  N  L  H  M  R  A  G

                GGAGGACGTGGTGCTCTGCGTGCTGCCGCTCTTCCACATCTTCTCGCTCAACTCGGTGCT
1081            ---------+---------+---------+---------+---------+---------+ 1140
                 E  D  V  V  L  C  V  L  P  L  F  H  I  F  S  L  N  S  V  L

                GCTGTGCGCGCTGCGGGCGGGCGCCGCCGTGATGCTGATGCCTAGGTTCGAGATGGGGGC
1141            ---------+---------+---------+---------+---------+---------+ 1200
                 L  C  A  L  R  A  G  A  A  V  M  L  M  P  R  F  E  M  G  A

                CATGCTGGAGGGCATCGAGCGGTGGCGCGTCACGGTGGCGGCCGTGGTGCCGCCGCTGGT
1201            ---------+---------+---------+---------+---------+---------+ 1260
                 M  L  E  G  I  E  R  W  R  V  T  V  A  A  V  V  P  P  L  V

                GCTCGCGCTCGCCAAGAACCCCGGGGTGGAGAAGCACGACCTCAGCTCCATTCGGATCGT
1261            ---------+---------+---------+---------+---------+---------+ 1320
                 L  A  L  A  K  N  P  G  V  E  K  H  D  L  S  S  I  R  I  V

                GCTCTCCGGCGCCGCGCCGCTCGGCAAGGAGCTCGAGGACGCGCTACGTGGCCGCCTGCC
1321            ---------+---------+---------+---------+---------+---------+ 1380
                 L  S  G  A  A  P  L  G  K  E  L  E  D  A  L  R  G  R  L  P

                GCAGGCCATCTTCGGACAGGGCTACGGGATGACGGAGGCCGGGCCGGTGCTGTCCATGTG
1381            ---------+---------+---------+---------+---------+---------+ 1440
                 Q  A  I  F  G  Q  G  Y  G  M  T  E  A  G  P  V  L  S  M  C

                CCCGGCGTTCGCGCGGGAGCCGACGCCGGCCAAGTCCGGCTCGTGCGGCACCGTGGTGCG
1441            ---------+---------+---------+---------+---------+---------+ 1500
                 P  A  F  A  R  E  P  T  P  A  K  S  G  S  C  G  T  V  V  R
```

FIGURE 2 CONTINUED

```
      CAACGCCCAGCTCAAGGTGGTCGACCCCGACACCGGCGTCTCCCTCGGCCGCAACCTCCC
1501  ---------+---------+---------+---------+---------+---------+  1560
       N  A  Q  L  K  V  V  D  P  D  T  G  V  S  L  G  R  N  L  P

      CGGCGAGATCTGCATCCGCGGCCCGCAGATCATGAAAGGATACTTGAATGATCCCGTGGC
1561  ---------+---------+---------+---------+---------+---------+  1620
       G  E  I  C  I  R  G  P  Q  I  M  K  G  Y  L  N  D  P  V  A

      CACGGCCGCGACCATCGACGTCGAGGGGTGGCTCCACACCGGCGACATCGGCTACGTCGA
1621  ---------+---------+---------+---------+---------+---------+  1680
       T  A  A  T  I  D  V  E  G  W  L  H  T  G  D  I  G  Y  V  D

      CGACGACGACGAGGTCTTCATCGTCGACCGCGTCAAGGAGCTCATCAAGTTCAAGGGCTT
1681  ---------+---------+---------+---------+---------+---------+  1740
       D  D  D  E  V  F  I  V  D  R  V  K  E  L  I  K  F  K  G  F

      CCAGGTACCGCCGGCCGAGCTCGAGGCTCTGCTCATCGCGCATCCGTCCATCGCCGACGC
1741  ---------+---------+---------+---------+---------+---------+  1800
       Q  V  P  P  A  E  L  E  A  L  L  I  A  H  P  S  I  A  D  A

      GGCCGTCGTCCCGCAAAAGGATGATGCCGCCGGCGAGGTCCCGGTTGCCTTCGTGGTCCG
1801  ---------+---------+---------+---------+---------+---------+  1860
       A  V  V  P  Q  K  D  D  A  A  G  E  V  P  V  A  F  V  V  R

      CGCCGCCGACTCCGACATCGCCGAGGAGGCCATCAAGGAGTTCGTATCCAAGCAGGTGGT
1861  ---------+---------+---------+---------+---------+---------+  1920
       A  A  D  S  D  I  A  E  E  A  I  K  E  F  V  S  K  Q  V  V

      GTTCTACAAGAGGCTGCACAAGGTCTACTTCACCCACGCGATACCCAAGTCGGCGTCGGG
1921  ---------+---------+---------+---------+---------+---------+  1980
       F  Y  K  R  L  H  K  V  Y  F  T  H  A  I  P  K  S  A  S  G

      GAAGATACTCAGGAAAGAACTCAGAGCTAAACTCGCCGCCCCGGCCACTGCCTGAAGAGT
1981  ---------+---------+---------+---------+---------+---------+  2040
       K  I  L  R  K  E  L  R  A  K  L  A  A  P  A  T  A  *  R  V

      GGTTCATGGCTTCATGCTAATCATTTCGATCAGAAAGGCACTTCTAGCATATATGTTCCA
2041  ---------+---------+---------+---------+---------+---------+  2100
       V  H  G  F  M  L  I  I  S  I  R  K  A  L  L  A  Y  M  F  H

      CCTTTTGTTTCATTTGGAAGATTGTATTCCAGCTAGTGGCCAGTGACTGAGTAAGGGATG
2101  ---------+---------+---------+---------+---------+---------+  2160
       L  L  F  H  L  E  D  C  I  P  A  S  G  Q  *

      GGGATAAAAGTTTTGTCTACGTTTTCTTTTACGCTACTCTCTCCATTGGGGAGTACAATG
2161  ---------+---------+---------+---------+---------+---------+  2220

      TATCAGGGGATTCGTGATTGAAGTTAATCAAGATTGGTTCAATTATAAAAAAAAAAAAAA
2221  ---------+---------+---------+---------+---------+---------+  2280

      AAAA
2281  ----  2284
```

FIGURE 2 CONTINUED

```
                CGGCACGAGCGCCATTCCTCCACCTTCAGCTCCGGCCAAAGATTTCCATCCGGCGAGATC
   1            ---------+---------+---------+---------+---------+---------+   60

                CATGGGCTCCATCGCGGCGGACGCGCCTCCCGCGGAGCTGGTGTTCCGGTCCAAGCTCCC
  61            ---------+---------+---------+---------+---------+---------+  120
                 M  G  S  I  A  A  D  A  P  P  A  E  L  V  F  R  S  K  L  P

                GGACATCGAGATCCCGACCCACCTGACGCTGCAGGACTACTGCTTCCAGCGCCTGCCGGA
 121            ---------+---------+---------+---------+---------+---------+  180
                 D  I  E  I  P  T  H  L  T  L  Q  D  Y  C  F  Q  R  L  P  E

                GCTCTCCGCGCGCGCCTGCCTCATCGACGGCGCCACGGGCGCCGCGCTCACCTACGGCGA
 181            ---------+---------+---------+---------+---------+---------+  240
                 L  S  A  R  A  C  L  I  D  G  A  T  G  A  A  L  T  Y  G  E

                GGTGGACGCCCTGTCCCGCCGCTGCGCCGCGGGGCTGCGCCGCCTCGGCGTCGGCAAGGG
 241            ---------+---------+---------+---------+---------+---------+  300
                 V  D  A  L  S  R  R  C  A  A  G  L  R  R  L  G  V  G  K  G

                CGACGTCGTCATGGCGCTCCTCCGCAACTGCCCCGAGTTCGCCTTCGTGTTCCTCGGCGC
 301            ---------+---------+---------+---------+---------+---------+  360
                 D  V  V  M  A  L  L  R  N  C  P  E  F  A  F  V  F  L  G  A

                GGCCCGGCTCGGCGCCGCCACCACCACCGCCAACCCGTTCTACACGCCCCACGAGATCCA
 361            ---------+---------+---------+---------+---------+---------+  420
                 A  R  L  G  A  A  T  T  T  A  N  P  F  Y  T  P  H  E  I  H

                CCGCCAGGCCACCGCCGCCGGGGCCAGGGTCATCGTCACCGAGGCCTGCGCCGTCGAGAA
 421            ---------+---------+---------+---------+---------+---------+  480
                 R  Q  A  T  A  A  G  A  R  V  I  V  T  E  A  C  A  V  E  K

                GGTGCGCGCCTTCGCCGCCGAGAGAGGGATTCCCGTCGTCTCCGTCGACGAGGGCGTCGA
 481            ---------+---------+---------+---------+---------+---------+  540
                 V  R  A  F  A  A  E  R  G  I  P  V  V  S  V  D  E  G  V  D

                CGGCGGCTGCCTCCCGTTCGCCGAGACTCTGCTCGGGGAAGAAAGCGGGGAGCGGTTCGT
 541            ---------+---------+---------+---------+---------+---------+  600
                 G  G  C  L  P  F  A  E  T  L  L  G  E  E  S  G  E  R  F  V

                CGACGAGGCGGTCGACCCCGACGACGTGGTGGCGCTGCCGTACTCGTCCGGCACCACCGG
 601            ---------+---------+---------+---------+---------+---------+  660
                 D  E  A  V  D  P  D  D  V  V  A  L  P  Y  S  S  G  T  T  G

                CCTGCCCAAGGGCGTCATGCTCACCCACCGCAGCCTCGTCACCAGCGTCGCCCAGCAGGT
 661            ---------+---------+---------+---------+---------+---------+  720
                 L  P  K  G  V  M  L  T  H  R  S  L  V  T  S  V  A  Q  Q  V

                GGACGGTGAGAACCCGAACCTGCACTTCAGCTCGTCGGACGTGCTGCTGTGCGTGCTGCC
 721            ---------+---------+---------+---------+---------+---------+  780
                 D  G  E  N  P  N  L  H  F  S  S  S  D  V  L  L  C  V  L  P
```

FIGURE 3

```
                GCTGTTCCACATCTACTCGCTCAACTCGGTGCTGCTCGCCGGTCTCCGCGCCGGGTGCGC
781             ---------+---------+---------+---------+---------+---------+  840
                 L  F  H  I  Y  S  L  N  S  V  L  L  A  G  L  R  A  G  C  A

                GATCGTGATCATGCGCAAGTTCGACCACGGCGCGCTGGTGGACCTGGTGCGCACGCACGG
841             ---------+---------+---------+---------+---------+---------+  900
                 I  V  I  M  R  K  F  D  H  G  A  L  V  D  L  V  R  T  H  G

                CGTCACCGTGGCGCCATTCGTGCCGCCCATCGTGGTGGAGATCGCCAAGAGCGCGCGGGT
901             ---------+---------+---------+---------+---------+---------+  960
                 V  T  V  A  P  F  V  P  P  I  V  V  E  I  A  K  S  A  R  V

                GACCGCCGCGGACCTGGCGTCCATCCGGCTGGTCATGTCGGGGGCGGCGCCCATGGGCAA
961             ---------+---------+---------+---------+---------+---------+ 1020
                 T  A  A  D  L  A  S  I  R  L  V  M  S  G  A  A  P  M  G  K

                GGAGCTGCAGGACGCGTTCATGGCCAAGATCCCCAACGCCGTGCTCGGCCAGGGATATGG
1021            ---------+---------+---------+---------+---------+---------+ 1080
                 E  L  Q  D  A  F  M  A  K  I  P  N  A  V  L  G  Q  G  Y  G

                GATGACCGAGGCCGGCCCTGTGCTGGCGATGTGCCTGGCCTTCGCCAAGGAGCCGTTCGC
1081            ---------+---------+---------+---------+---------+---------+ 1140
                 M  T  E  A  G  P  V  L  A  M  C  L  A  F  A  K  E  P  F  A

                GGTCAAGTCCGGTTCCTGCGGCACCGTCGTCAGGAACGCCGAGCTCAAGATCGTCGACCC
1141            ---------+---------+---------+---------+---------+---------+ 1200
                 V  K  S  G  S  C  G  T  V  V  R  N  A  E  L  K  I  V  D  P

                CGACACCGGCGCCTCCCTCGGCCGCAACCTGCCGGGGGAGATCTGCATCCGCGGCAAGCA
1201            ---------+---------+---------+---------+---------+---------+ 1260
                 D  T  G  A  S  L  G  R  N  L  P  G  E  I  C  I  R  G  K  Q

                GATCATGAAAGGTTACCTAAATGATCCGGTGGCCACAAAGAACACCATTGACAAGGACGG
1261            ---------+---------+---------+---------+---------+---------+ 1320
                 I  M  K  G  Y  L  N  D  P  V  A  T  K  N  T  I  D  K  D  G

                TTGGCTGCATACTGGTGACATTGGTTATGTCGATGATGACGACGAGATCTTTATTGTCGA
1321            ---------+---------+---------+---------+---------+---------+ 1380
                 W  L  H  T  G  D  I  G  Y  V  D  D  D  D  E  I  F  I  V  D

                CAGACTGAAGGAGATAATTAAATATAAGGGATTCCAAGTACCTCCGGCGGAACTTGAAGC
1381            ---------+---------+---------+---------+---------+---------+ 1440
                 R  L  K  E  I  I  K  Y  K  G  F  Q  V  P  P  A  E  L  E  A

                CCTTCTCATTACACACCCTGAAATCAAGGATGCTGCTGTCGTATCGATGCAAGACGAACT
1441            ---------+---------+---------+---------+---------+---------+ 1500
                 L  L  I  T  H  P  E  I  K  D  A  A  V  V  S  M  Q  D  E  L

                TGCTGGTGAAGTTCCGGTTGCGTTTGTTGTGCGGACTGAGGGTTCAGAGATCAGCGAAAA
1501            ---------+---------+---------+---------+---------+---------+ 1560
                 A  G  E  V  P  V  A  F  V  V  R  T  E  G  S  E  I  S  E  N
```

FIGURE 3 CONTINUED

```
      CGAGATCAAGCAGTTCGTTGCAAAAGAGGTTGTTTTCTACAAGAGGATCTGCAAAGTGTT
1561  ---------+---------+---------+---------+---------+---------+  1620
       E  I  K  Q  F  V  A  K  E  V  V  F  Y  K  R  I  C  K  V  F

      CTTCGCGGATTCCATTCCAAAGAGTCCATCTGGCAAGATCCTCAGGAAGGACCTGAGAGC
1621  ---------+---------+---------+---------+---------+---------+  1680
       F  A  D  S  I  P  K  S  P  S  G  K  I  L  R  K  D  L  R  A

      AAAGCTCGCCGCAGGCATTCCCAGCAGTAATACCACACAGTCCAAAAGCTAAGTCAGATA
1681  ---------+---------+---------+---------+---------+---------+  1740
       K  L  A  A  G  I  P  S  S  N  T  T  Q  S  K  S  *

      TATTGTTTCCCAACCTTACACACCTCTGTCCAACACCATGTAATGTTCTTAATATAAACG
1741  ---------+---------+---------+---------+---------+---------+  1800

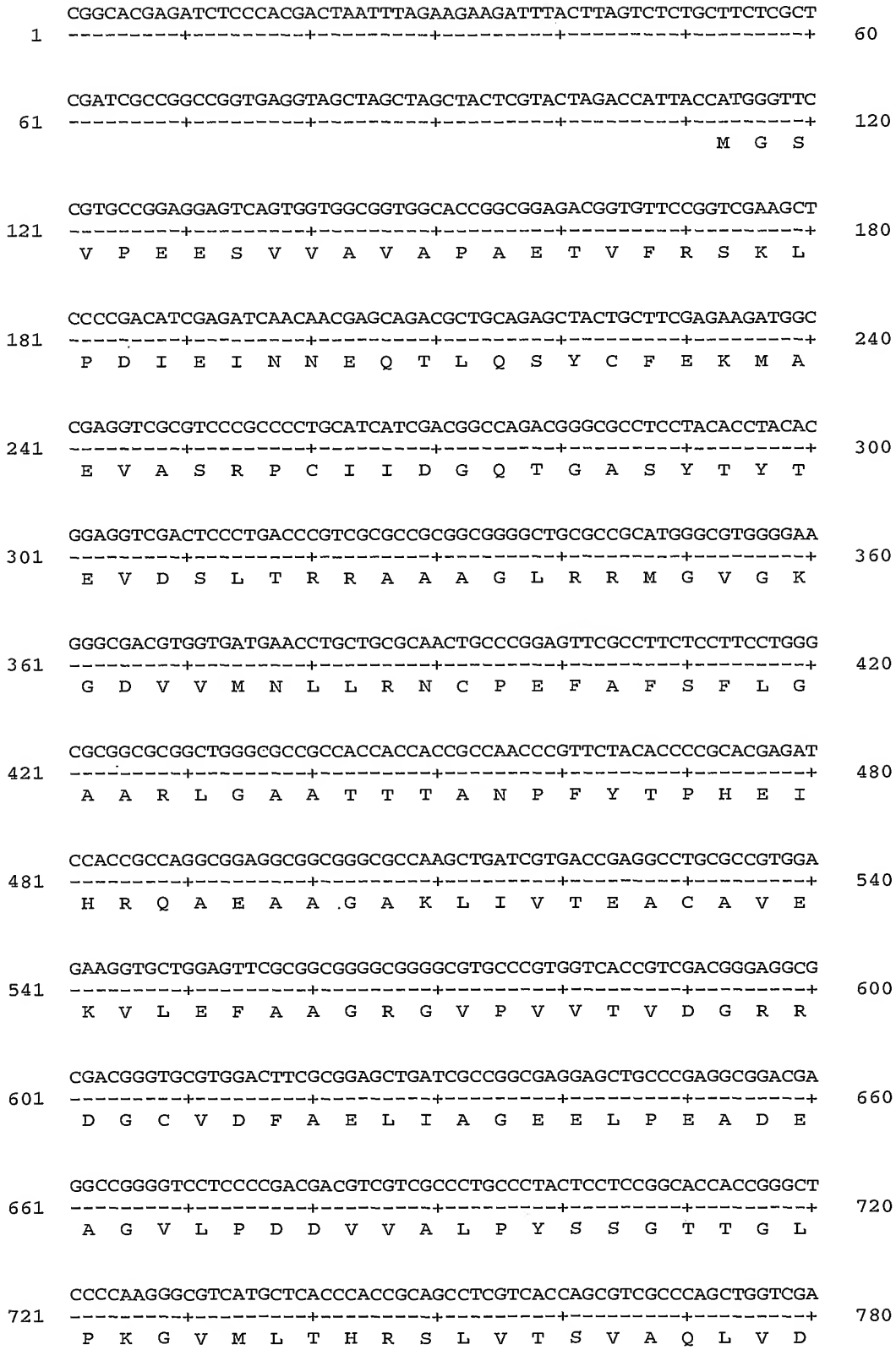
      GAAATTATTACATATAGAAGGGCTGATTCTTTTTACTAGATGTGTCCAACATATGATATG
1801  ---------+---------+---------+---------+---------+---------+  1860

      CTTGTTAGGCCGATGATGTGTAACCTGTCATGTATAGATACCGCCTTTTTTTGACAAGAA
1861  ---------+---------+---------+---------+---------+---------+  1920

      AGGCTGATTATAATGTATACCGTGAACTGAATATTTGTTCAGGGAGATCAAAAAAAAAAA
1921  ---------+---------+---------+---------+---------+---------+  1980

      AAAAAAAAAAAA
1981  ---------+--  1992
```

FIGURE 3 CONTINUED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CGATCGCCGGCCGGTGAGGTAGCTAGCTAGCTACTCGTACTAGACCATTACCATGGGTTC
61 120
M G S
CGTGCCGGAGGAGTCAGTGGTGGCGGTGGCACCGGCGGAGACGGTGTTCCGGTCGAAGCT
121 180
V P E E S V V A V A P A E T V F R S K L
CCCCGACATCGAGATCAACAACGAGCAGACGCTGCAGAGCTACTGCTTCGAGAAGATGGC
181 240
P D I E I N N E Q T L Q S Y C F E K M A
CGAGGTCGCGTCCCGCCCCTGCATCATCGACGGCCAGACGGGCGCCTCCTACACCTACAC
241 300
E V A S R P C I I D G Q T G A S Y T Y T
GGAGGTCGACTCCCTGACCCGTCGCGCCGCGGCGGGGCTGCGCCGCATGGGCGTGGGGAA
301 360
E V D S L T R R A A A G L R R M G V G K
GGGCGACGTGGTGATGAACCTGCTGCGCAACTGCCCGGAGTTCGCCTTCTCCTTCCTGGG
361 420
G D V V M N L L R N C P E F A F S F L G
CGCGGCGCGGCTGGGCGCCGCCACCACCACCGCCAACCCGTTCTACACCCCGCACGAGAT
421 480
A A R L G A A T T T A N P F Y T P H E I
CCACCGCCAGGCGGAGGCGGCGGGCGCCAAGCTGATCGTGACCGAGGCCTGCGCCGTGGA
481 540
H R Q A E A A G A K L I V T E A C A V E
GAAGGTGCTGGAGTTCGCGGCGGGGCGGGGCGTGCCCGTGGTCACCGTCGACGGGAGGCG
541 600
K V L E F A A G R G V P V V T V D G R R
CGACGGGTGCGTGGACTTCGCGGAGCTGATCGCCGGCGAGGAGCTGCCCGAGGCGGACGA
601 660
D G C V D F A E L I A G E E L P E A D E
GGCCGGGGTCCTCCCCGACGACGTCGTCGCCCTGCCCTACTCCTCCGGCACCACCGGGCT
661 720
A G V L P D D V V A L P Y S S G T T G L
CCCCAAGGGCGTCATGCTCACCCACCGCAGCCTCGTCACCAGCGTCGCCCAGCTGGTCGA
721 780
P K G V M L T H R S L V T S V A Q L V D

FIGURE 4

```
                CGGGTCGAACCCTAACGTGTGCTTCAACAAGGACGACGCGCTGCTGTGCCTGCTGCCGCT
781             ---------+---------+---------+---------+---------+---------+ 840
                 G  S  N  P  N  V  C  F  N  K  D  D  A  L  L  C  L  L  P  L

                CTTCCACATCTACTCGCTGCACACGGTGCTGCTGGCGGGGCTCCGCGTCGGCGCCGCCAT
841             ---------+---------+---------+---------+---------+---------+ 900
                 F  H  I  Y  S  L  H  T  V  L  L  A  G  L  R  V  G  A  A  I

                CGTCATCATGCGCAAGTTCGACGTCGGCGCGCTGGTGGACCTCGTCCGCGCGCACCGCAT
901             ---------+---------+---------+---------+---------+---------+ 960
                 V  I  M  R  K  F  D  V  G  A  L  V  D  L  V  R  A  H  R  I

                CACCATCGCGCCATTCGTGCCGCCCATCGTCGTGGAGATCGCCAAGAGCGACCGCGTCGG
961             ---------+---------+---------+---------+---------+---------+ 1020
                 T  I  A  P  F  V  P  P  I  V  V  E  I  A  K  S  D  R  V  G

                CGCCGACGACCTCGCATCCATCCGCATGGTGCTCTCCGGCGCCGCGCCCATGGGCAAGGA
1021            ---------+---------+---------+---------+---------+---------+ 1080
                 A  D  D  L  A  S  I  R  M  V  L  S  G  A  A  P  M  G  K  D

                CCTCCAGGACGCCTTCATGGCCAAGATCCCCAACGCCGTGCTCGGACAGGGGTACGGGAT
1081            ---------+---------+---------+---------+---------+---------+ 1140
                 L  Q  D  A  F  M  A  K  I  P  N  A  V  L  G  Q  G  Y  G  M

                GACCGAGGCTGGGCCGGTGCTGGCCATGTGCCTGGCGTTCGCCAAGGAGCCGTTCAAGGT
1141            ---------+---------+---------+---------+---------+---------+ 1200
                 T  E  A  G  P  V  L  A  M  C  L  A  F  A  K  E  P  F  K  V

                CAAGTCCGGGTCGTGCGGAACCGTGGTGCGCAACGCCGAGCTCAAGGTCGTCGACCCCGA
1201            ---------+---------+---------+---------+---------+---------+ 1260
                 K  S  G  S  C  G  T  V  V  R  N  A  E  L  K  V  V  D  P  D

                CACCGGCGCATCCCTCGGCCGGAACCAGCCTGGCGAGATTTGCGTCCGGGGGAAGCAGAT
1261            ---------+---------+---------+---------+---------+---------+ 1320
                 T  G  A  S  L  G  R  N  Q  P  G  E  I  C  V  R  G  K  Q  I

                CATGATAGGTTACCTGAACGACCCAGAGTCGACCAAGAACACCATCGACAAGGACGGCTG
1321            ---------+---------+---------+---------+---------+---------+ 1380
                 M  I  G  Y  L  N  D  P  E  S  T  K  N  T  I  D  K  D  G  W

                GCTGCACACCGGAGACATCGGCTTGGTGGATGACGACGACGAGATCTTCATCGTCGACAG
1381            ---------+---------+---------+---------+---------+---------+ 1440
                 L  H  T  G  D  I  G  L  V  D  D  D  D  E  I  F  I  V  D  R

                GCTCAAGGAGATCATCAAGTACAAGGGCTTCCAAGTGGCGCCGGCGGAGCTCGAGGCCCT
1441            ---------+---------+---------+---------+---------+---------+ 1500
                 L  K  E  I  I  K  Y  K  G  F  Q  V  A  P  A  E  L  E  A  L

                CCTCCTCACGAACCCGGAGGTCAAGGACGCCGCCGTCGTAGGGGTGAAGGATGATCTCTG
1501            ---------+---------+---------+---------+---------+---------+ 1560
                 L  L  T  N  P  E  V  K  D  A  A  V  V  G  V  K  D  D  L  C
```

FIGURE 4 CONTINUED

```
      CGGCGAAGTCCCGGTCGCCTTCATTAAGAGGATCGAAGGATCTGAGATCAACGAGAACGA
1561  ---------+---------+---------+---------+---------+---------+  1620
       G  E  V  P  V  A  F  I  K  R  I  E  G  S  E  I  N  E  N  E

      GATCAAGCAATTCGTCTCAAAGGAGGTTGTTTTCTACAAGAGGATCAACAAGGTCTACTT
1621  ---------+---------+---------+---------+---------+---------+  1680
       I  K  Q  F  V  S  K  E  V  V  F  Y  K  R  I  N  K  V  Y  F

      CACCGACTCCATTCCCAAGAACCCTTCCGGCAAGATCCTAAGGAAGGACTTGAGAGCCAG
1681  ---------+---------+---------+---------+---------+---------+  1740
       T  D  S  I  P  K  N  P  S  G  K  I  L  R  K  D  L  R  A  R

      GCTCGCCGCTGGCATCCCCACCGAAGTTGCCGCGCCGAGAAGCTAAGGGCCGCTTCTCAG
1741  ---------+---------+---------+---------+---------+---------+  1800
       L  A  A  G  I  P  T  E  V  A  A  P  R  S  *

      GAACGCAGTCACCCATGGTGCTGTTTAGGTGCTGTTATAGACCACACCAAATGGGGAAAG
1801  ---------+---------+---------+---------+---------+---------+  1860

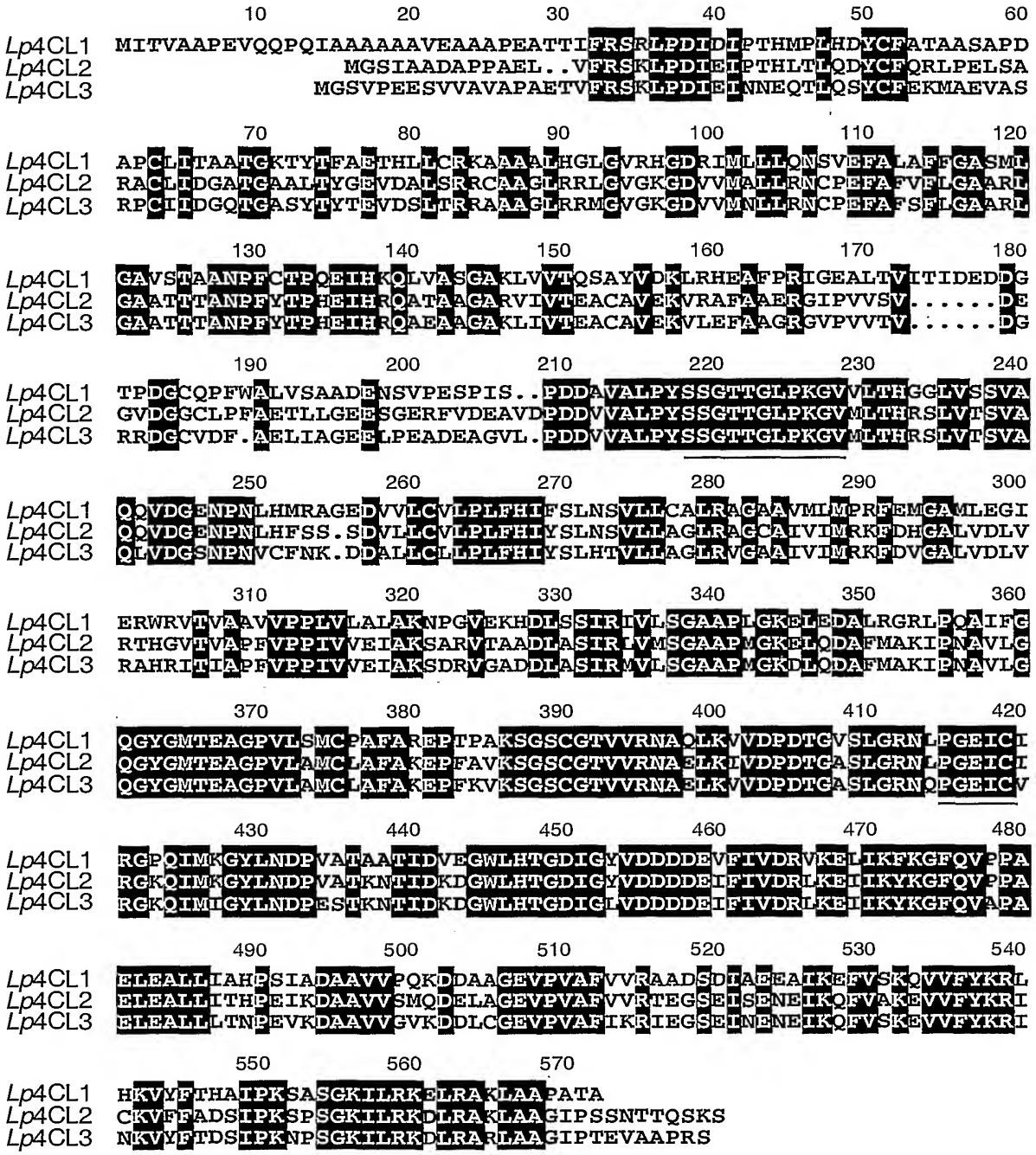
      AAACTACGGGAGGGGATCATATTATTGTTGCAGGAGATATCAGTTTGTTGATTCGCCCTG
1861  ---------+---------+---------+---------+---------+---------+  1920

      CTTGTGTAATGTTGATAAAATGAAATGATATAATAGATGTGTTGTTTTATTTTTTGACCA
1921  ---------+---------+---------+---------+---------+---------+  1980

      TGTAAGAACAAGGCTGTTTTATACACTACTTATTTTTTGAAAAAAAAAAAAAAAAAAA
1981  ---------+---------+---------+---------+---------+--------  2038
```

FIGURE 4 CONTINUED

FIGURE 5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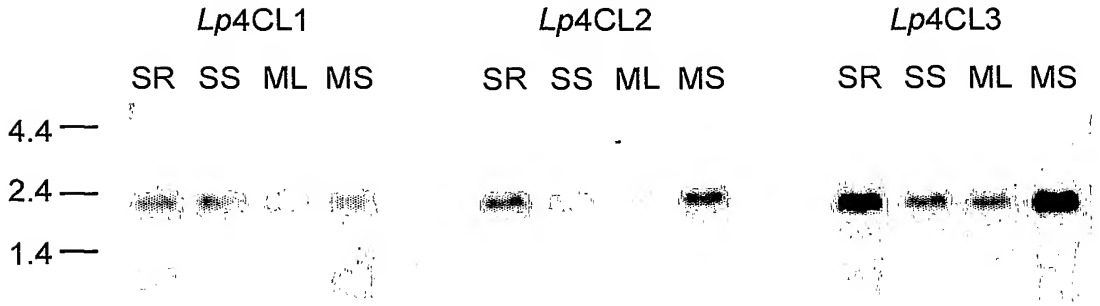
Lp4CL2 MGSIAADAPPAEL..VFRSKLPDIEIPTHLTLQDYCFQRLPELSA
Lp4CL3 MGSVPEESVVAVAPAETVFRSKLPDIEINNEQTLQSYCFEKMAEVAS
70 80 90 100 110 120
Lp4CL1 APCLITAATGKTYTFAETHLLCRKAAAALHGLGVRHGDRIMLLLQNSVEFALAFFGASML
Lp4CL2 RACLIDGATGAALTYGEVDALSRRCAAGLRRLGVGKGDVVMALLRNCPEFAFVFLGAARL
Lp4CL3 RPCIIDGQTGASYTYTEVDSLTRRAAAGLRRMGVGKGDVVMNLLRNCPEFAFSFLGAARL
130 140 150 160 170 180
Lp4CL1 GAVSTAANPFCTPQEIHKQLVASGAKLVVTQSAYVDKLRHEAFPRIGEALTVITIDEDDG
Lp4CL2 GAATTTANPFYTPHEIHRQATAAGARVIVTEACAVEKVRAFAAERGIPVVSV.....DE
Lp4CL3 GAATTTANPFYTPHEIHRQAEAAGAKLIVTEACAVEKVLEFAAGRGVPVVTV.....DG
190 200 210 220 230 240
Lp4CL1 TPDGCQPFWALVSAADENSVPESPIS..PDDAVALPYSSGTTGLPKGVVLTHGGLVSSVA
Lp4CL2 GVDGGCLPFAETLLGEESGERFVDEAVDPDDVVALPYSSGTTGLPKGVMLTHRSLVTSVA
Lp4CL3 RRDGCVDF.AELIAGEELPEADEAGVL.PDDVVALPYSSGTTGLPKGVMLTHRSLVTSVA
250 260 270 280 290 300
Lp4CL1 QQVDGENPNLHMRAGEDVVLCVLPLFHIFSLNSVLLCALRAGAAVMLMPRFEMGAMLEGI
Lp4CL2 QQVDGENPNLHFSS.SDVLLCVLPLFHIYSLNSVLLAGLRAGCAIVIMRKFDHGALVDLV
Lp4CL3 QLVDGSNPNVCFNK.DDALLCLLPLFHIYSLHTVLLAGLRVGAAIVIMRKFDVGALVDLV
310 320 330 340 350 360
Lp4CL1 ERWRVTVAAVVPPLVLALAKNPGVEKHDLSSIRIVLSGAAPLGKELEDALRGRLPQAIFG
Lp4CL2 RTHGVTVAPFVPPIVVEIAKSARVTAADLASIRIVMSGAAPMGKELQDAFMAKIPNAVLG
Lp4CL3 RAHRITIAPFVPPIVVEIAKSDRVGADDLASIRMVLSGAAPMGKDLQDAFMAKIPNAVLG
370 380 390 400 410 420
Lp4CL1 QGYGMTEAGPVLSMCLAFAKEPFPVKSGSCGTVVRNAQLKVVDPDTGVSLGRNLPGEICI
Lp4CL2 QGYGMTEAGPVLAMCLAFAKEPFAVKSGSCGTVVRNAELKIVDPDTGASLGRNLPGEICI
Lp4CL3 QGYGMTEAGPVLAMCLAFAKEPFKVKSGSCGTVVRNAELKVVDPDTGASLGRNQPGEICV
430 440 450 460 470 480
Lp4CL1 RGPQIMKGYLNDPVATAATIDVEGWLHTGDIGYVDDDDEVFIVDRVKELIKFKGFQVPPA
Lp4CL2 RGKQIMKGYLNDPVATKNTIDKDGWLHTGDIGYVDDDDEIFIVDRLKEIIKYKGFQVPPA
Lp4CL3 RGKQIMIGYLNDPESTKNTIDKDGWLHTGDIGLVDDDDEIFIVDRLKEIIKYKGFQVAPA
490 500 510 520 530 540
Lp4CL1 ELEALLIAHPSIADAAVVPQKDDAAGEVPVAFVVRAADSDIAEEAIKEFVSKQVVFYKRL
Lp4CL2 ELEALLITHPEIKDAAVVSMQDELAGEVPVAFVVRTEGSEISENEIKQFVAKEVVFYKRI
Lp4CL3 ELEALLLTNPEVKDAAVVGVKDDLCGEVPVAFIKRIEGSEINENEIKQFVSKEVVFYKRI
550 560 570
Lp4CL1 HKVYFTHAIPKSASGKILRKELRAKLAAPATA
Lp4CL2 CKVFFADSIPKSPSGKILRKDLRAKLAAGIPSSNTTQSKS
Lp4CL3 NKVYFTDSIPKNPSGKILRKDLRARLAAGIPTEVAAPRS

FIGURE 6



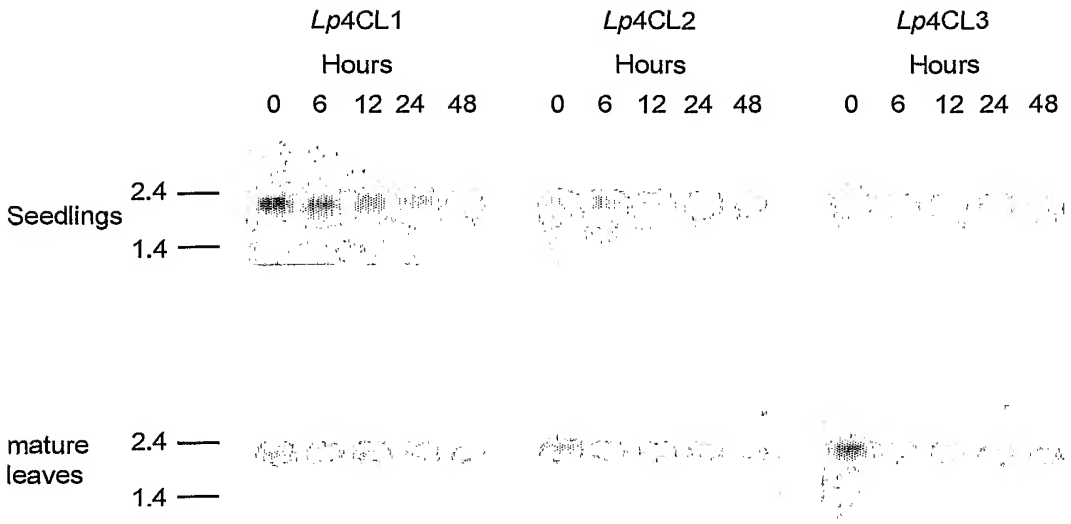

Lp4CL1
Hours
0 6 12 24 48
Lp4CL2
Hours
0 6 12 24 48
Lp4CL3
Hours
0 6 12 24 48
Seedlings
2.4
1.4
mature leaves
2.4
1.4

FIGURE 7

FIGURE 8

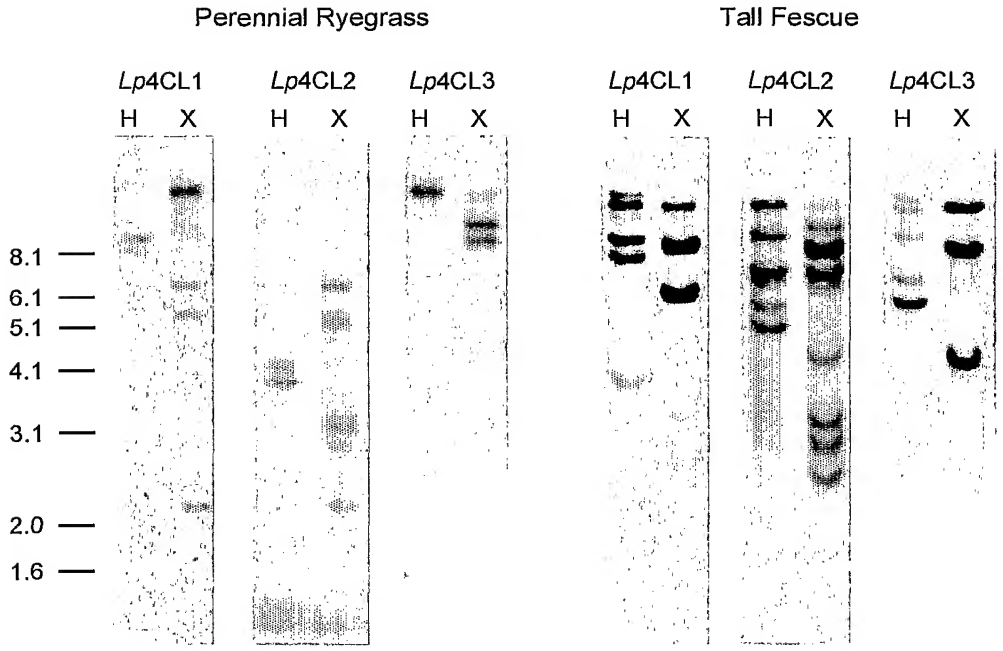
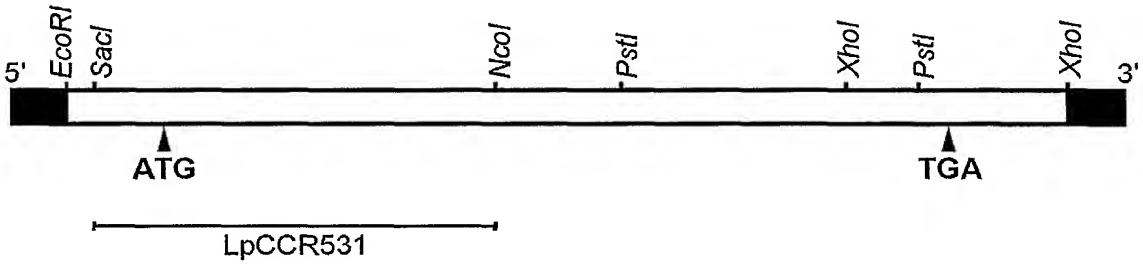

Perennial Ryegrass
Tall Fescue
Lp4CL1
Lp4CL2
Lp4CL3
H X
8.1
6.1
5.1
4.1
3.1
2.0
1.6

FIGURE 9



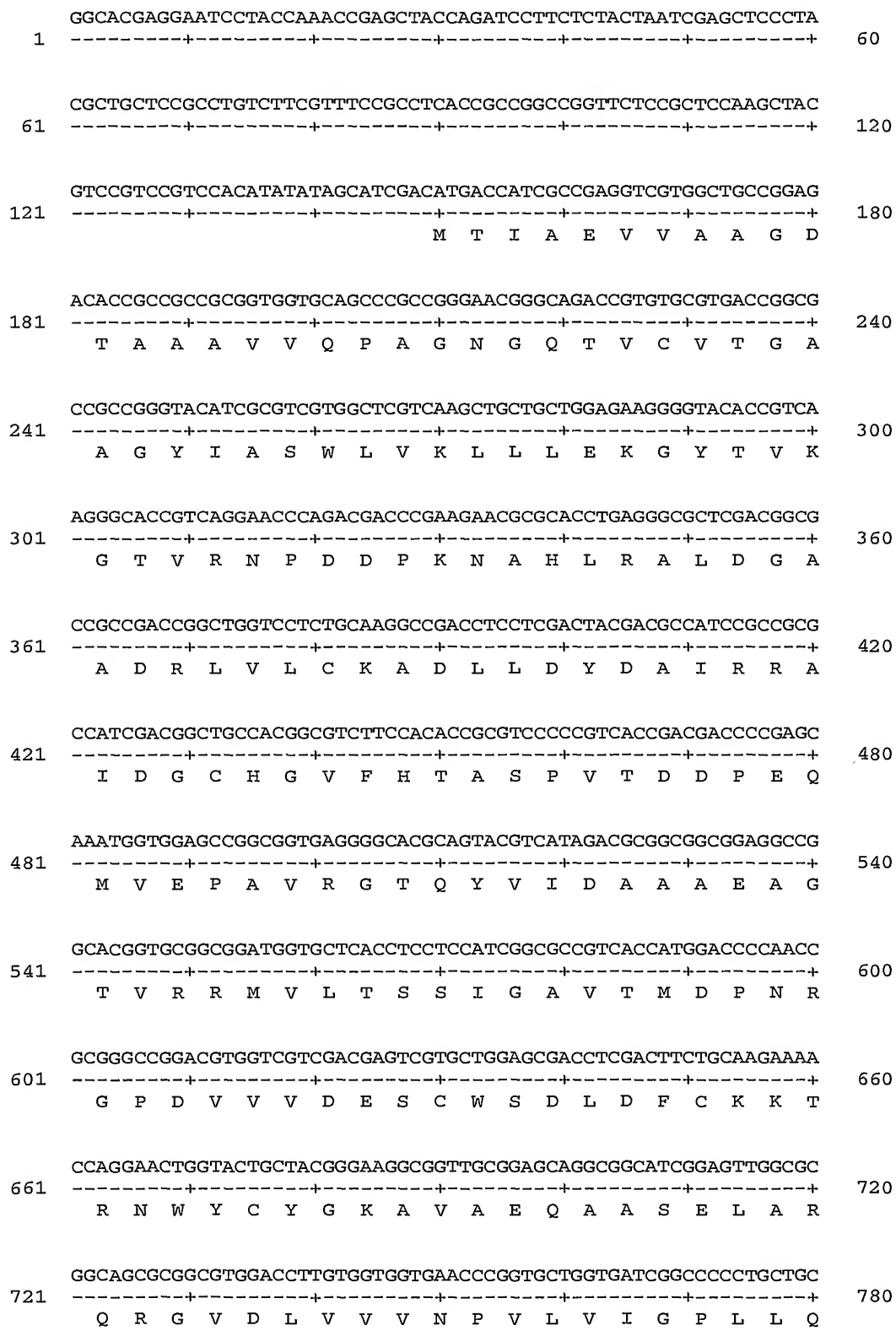

GGCACGAGGAATCCTACCAAACCGAGCTACCAGATCCTTCTCTACTAATCGAGCTCCCTA
1 60
CGCTGCTCCGCCTGTCTTCGTTTCCGCCTCACCGCCGGCCGGTTCTCCGCTCCAAGCTAC
61 120
GTCCGTCCGTCCACATATATAGCATCGACATGACCATCGCCGAGGTCGTGGCTGCCGGAG
121 180
M T I A E V V A A G D
ACACCGCCGCCGCGGTGGTGCAGCCCGCCGGGAACGGGCAGACCGTGTGCGTGACCGGCG
181 240
T A A A V V Q P A G N G Q T V C V T G A
CCGCCGGGTACATCGCGTCGTGGCTCGTCAAGCTGCTGCTGGAGAAGGGGTACACCGTCA
241 300
A G Y I A S W L V K L L L E K G Y T V K
AGGGCACCGTCAGGAACCCAGACGACCCGAAGAACGCGCACCTGAGGGCGCTCGACGGCG
301 360
G T V R N P D D P K N A H L R A L D G A
CCGCCGACCGGCTGGTCCTCTGCAAGGCCGACCTCCTCGACTACGACGCCATCCGCCGCG
361 420
A D R L V L C K A D L L D Y D A I R R A
CCATCGACGGCTGCCACGGCGTCTTCCACACCGCGTCCCCCGTCACCGACGACCCCGAGC
421 480
I D G C H G V F H T A S P V T D D P E Q
AAATGGTGGAGCCGGCGGTGAGGGGCACGCAGTACGTCATAGACGCGGCGGCGGAGGCCG
481 540
M V E P A V R G T Q Y V I D A A A E A G
GCACGGTGCGGCGGATGGTGCTCACCTCCTCCATCGGCGCCGTCACCATGGACCCCAACC
541 600
T V R R M V L T S S I G A V T M D P N R
GCGGGCCGGACGTGGTCGTCGACGAGTCGTGCTGGAGCGACCTCGACTTCTGCAAGAAAA
601 660
G P D V V V D E S C W S D L D F C K K T
CCAGGAACTGGTACTGCTACGGGAAGGCGGTTGCGGAGCAGGCGGCATCGGAGTTGGCGC
661 720
R N W Y C Y G K A V A E Q A A S E L A R
GGCAGCGCGGCGTGGACCTTGTGGTGGTGAACCCGGTGCTGGTGATCGGCCCCCTGCTGC
721 780
Q R G V D L V V V N P V L V I G P L L Q

FIGURE 10

```
    AGCCGACGGTGAACGCCAGCATCGGCCACATCCTCAAGTACCTGGACGGGTCGGCCAGCA
781 ---------+---------+---------+---------+---------+---------+ 840
      P  T  V  N  A  S  I  G  H  I  L  K  Y  L  D  G  S  A  S  K

    AGTTCGCCAACGCCGTGCAGGCGTACGTGGACGTCCGCGACGTGGCCGACGCCCACCTCC
841 ---------+---------+---------+---------+---------+---------+ 900
      F  A  N  A  V  Q  A  Y  V  D  V  R  D  V  A  D  A  H  L  R

    GCGTCTTCGAGTGCGCCGCCGCGTCCGGCCGCCACCTCTGCGCCGAGCGCGTCCTCCACC
901 ---------+---------+---------+---------+---------+---------+ 960
      V  F  E  C  A  A  A  S  G  R  H  L  C  A  E  R  V  L  H  R

    GCGAGGACGTCGTGCGCATCCTCGCCAAGCTCTTCCCCGAGTACCCCGTCCCCACCAGGT
961 ---------+---------+---------+---------+---------+---------+ 1020
      E  D  V  V  R  I  L  A  K  L  F  P  E  Y  P  V  P  T  R  C

    GCTCTGATGAGACGAACCCGAGGAAGCAGCCATACAAGATGTCGAACCAGAAGCTCCAGG
1021 ---------+---------+---------+---------+---------+---------+ 1080
      S  D  E  T  N  P  R  K  Q  P  Y  K  M  S  N  Q  K  L  Q  D

    ACCTCGGACTCGAGTTCAGGCCGGTGAGCCAGTCCCTGTACGAGACGGTGAAGAGCCTCC
1081 ---------+---------+---------+---------+---------+---------+ 1140
      L  G  L  E  F  R  P  V  S  Q  S  L  Y  E  T  V  K  S  L  Q

    AGGAGAAGGGCCACCTTCCGGTGCTCAGCGAGCAGGCAGAGGCGGACAAGGAAACCCTAG
1141 ---------+---------+---------+---------+---------+---------+ 1200
      E  K  G  H  L  P  V  L  S  E  Q  A  E  A  D  K  E  T  L  A

    CTGCCGAGCTGCAGGCAGGGGTTACCATCCGAGCATGAGGAACAAGAAATCAACCATGTC
1201 ---------+---------+---------+---------+---------+---------+ 1260
      A  E  L  Q  A  G  V  T  I  R  A  *

    CATACTGCTACTGTCATGTAAACCAGCTGTTGAATGCCTAAAATCTAAGTTCTTGTAATA
1261 ---------+---------+---------+---------+---------+---------+ 1320

    CTGTGTTGTTTCATGTGGACTAGATTGATCGAATAAACATCTCTACACAAGGTTGCTAAA
1321 ---------+---------+---------+---------+---------+---------+ 1380

    AAAAAAAAAAAAAAA
1381 ---------+-----  1395
```

FIGURE 10 CONTINUED

FIGURE 11

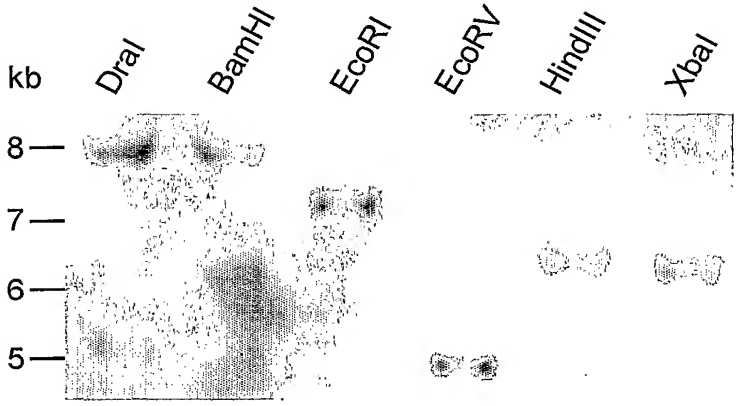
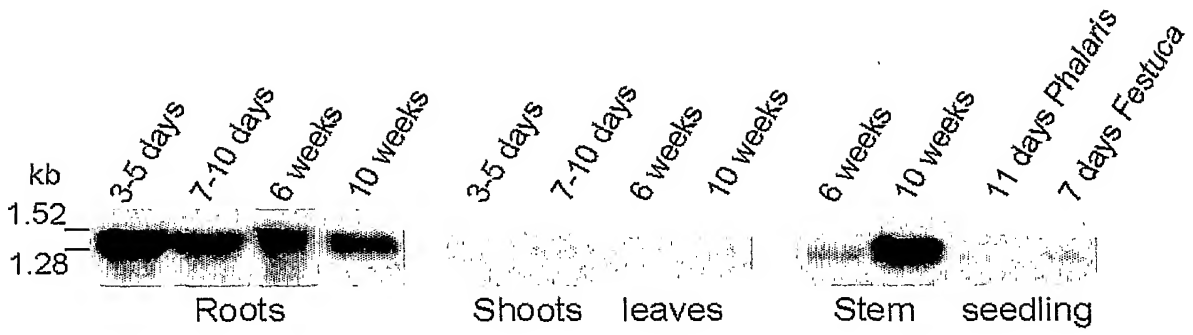


FIGURE 12


kb
1.52
1.28
3-5 days
7-10 days
6 weeks
10 weeks
3-5 days
7-10 days
6 weeks
10 weeks
6 weeks
10 weeks
11 days Phalaris
7 days Festuca
Roots
Shoots
leaves
Stem
seedling

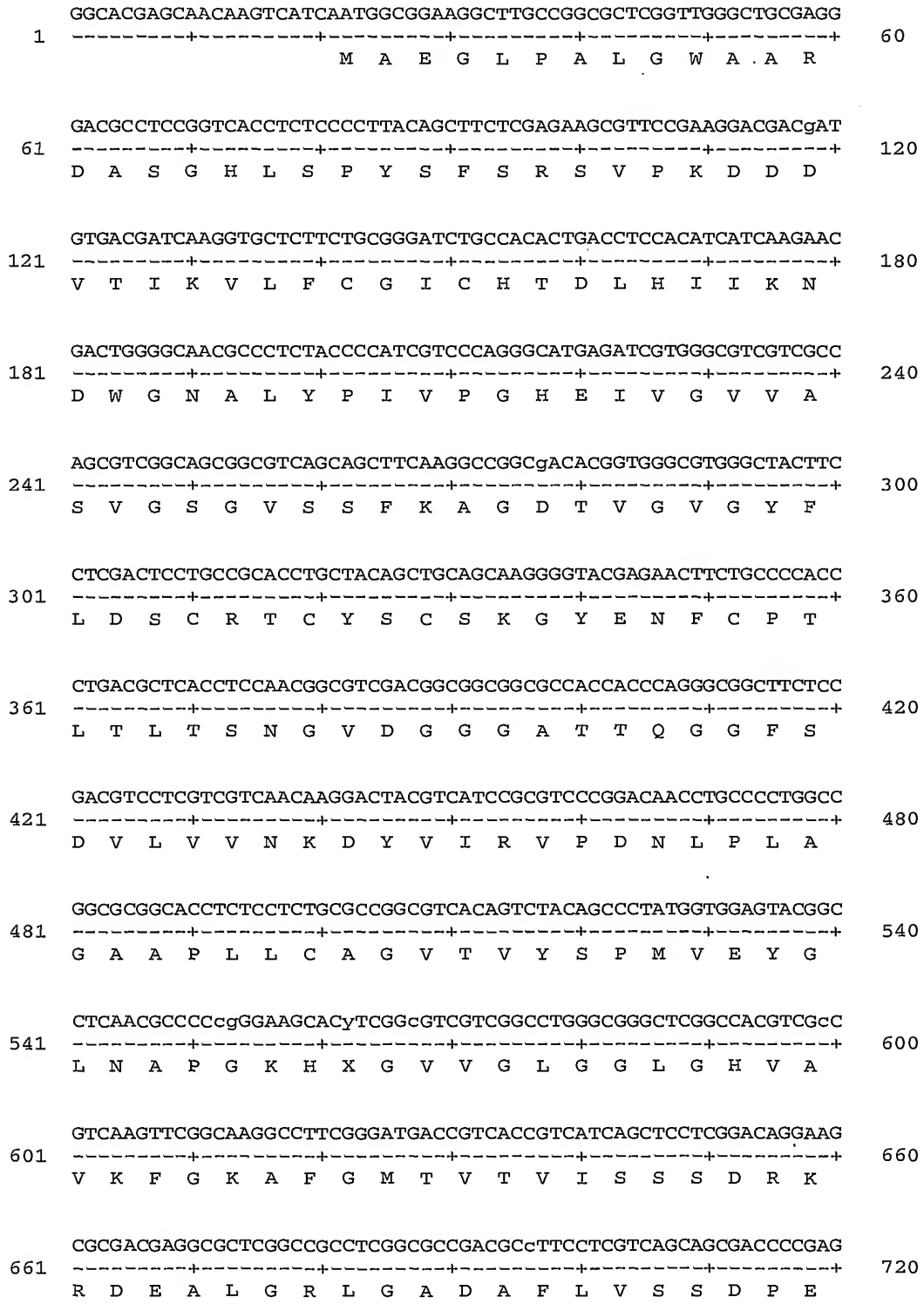

GGCACGAGCAACAAGTCATCAATGGCGGAAGGCTTGCCGGCGCTCGGTTGGGCTGCGAGG
1 60
M A E G L P A L G W A A R
GACGCCTCCGGTCACCTCTCCCCTTACAGCTTCTCGAGAAGCGTTCCGAAGGACGACgAT
61 120
D A S G H L S P Y S F S R S V P K D D D
GTGACGATCAAGGTGCTCTTCTGCGGGATCTGCCACACTGACCTCCACATCATCAAGAAC
121 180
V T I K V L F C G I C H T D L H I I K N
GACTGGGGCAACGCCCTCTACCCCATCGTCCCAGGGCATGAGATCGTGGGCGTCGTCGCC
181 240
D W G N A L Y P I V P G H E I V G V V A
AGCGTCGGCAGCGGCGTCAGCAGCTTCAAGGCCGGCgACACGGTGGGCGTGGGCTACTTC
241 300
S V G S G V S S F K A G D T V G V G Y F
CTCGACTCCTGCCGCACCTGCTACAGCTGCAGCAAGGGGTACGAGAACTTCTGCCCCACC
301 360
L D S C R T C Y S C S K G Y E N F C P T
CTGACGCTCACCTCCAACGGCGTCGACGGCGGCGGCGCCACCACCCAGGGCGGCTTCTCC
361 420
L T L T S N G V D G G G A T T Q G G F S
GACGTCCTCGTCGTCAACAAGGACTACGTCATCCGCGTCCCGGACAACCTGCCCCTGGCC
421 480
D V L V V N K D Y V I R V P D N L P L A
GGCGCGGCACCTCTCCTCTGCGCCGGCGTCACAGTCTACAGCCCTATGGTGGAGTACGGC
481 540
G A A P L L C A G V T V Y S P M V E Y G
CTCAACGCCCCcgGGAAGCACyTCGGcGTCGTCGGCCTGGGCGGGCTCGGCCACGTCGcC
541 600
L N A P G K H X G V V G L G G L G H V A
GTCAAGTTCGGCAAGGCCTTCGGGATGACCGTCACCGTCATCAGCTCCTCGGACAGGAAG
601 660
V K F G K A F G M T V T V I S S S D R K
CGCGACGAGGCGCTCGGCCGCCTCGGCGCCGACGCcTTCCTCGTCAGCAGCGACCCCGAG
661 720
R D E A L G R L G A D A F L V S S D P E

FIGURE 13

```
    CAGATGAAGGCGGCGGCGGGCACCATGGACGGCATCATCGACACGGTGTCCGCGGGCCAC
721 ---------+---------+---------+---------+---------+---------+ 780
    Q  M  K  A  A  A  G  T  M  D  G  I  I  D  T  V  S  A  G  H

    CCGATCGTGCCGCTGCTCGACCTGCTCAAGCCCATGGGGCAGATGGTCGTGGTGGGCGCG
781 ---------+---------+---------+---------+---------+---------+ 840
    P  I  V  P  L  L  D  L  L  K  P  M  G  Q  M  V  V  V  G  A

    CCCAGCAAGCCGCTCGAGCTCCCGGCCTTCGCCATCATCGGCGGCGGCAAGCGCCTCGCC
841 ---------+---------+---------+---------+---------+---------+ 900
    P  S  K  P  L  E  L  P  A  F  A  I  I  G  G  G  K  R  L  A

    GGGAGCGGCACCGGCAGCGTCGCACACTGCCagGCCATGCTCGACTTCGCGGGCAAGCAC
901 ---------+---------+---------+---------+---------+---------+ 960
    G  S  G  T  G  S  V  A  H  C  Q  A  M  L  D  F  A  G  K  H

    GGCATCACCGCCGACGTCGAGGTCGTCAAGATGGACTACgGTCAACACCGCCATCGAGCG
961 ---------+---------+---------+---------+---------+---------+ 1020
    G  I  T  A  D  V  E  V  V  K  M  D  Y  G  Q  H  R  H  R  A

    GCTAGAGAAGAACGACGTCAGGTACCGCTTCGTCATCGACGTCGCCGGCAGCCACCTGCA
1021---------+---------+---------+---------+---------+---------+ 1080
    A  R  E  E  R  R  Q  V  P  L  R  H  R  R  R  R  Q  P  P  A

    GGGCACCGCCGCTTAACTTGTGCTACACAATGTGGACGCGCGCTCGTTTGGTCCAGAAAA
1081---------+---------+---------+---------+---------+---------+ 1140
    G  H  R  R  L  T  C  A  T  Q  C  G  R  A  L  V  W  S  R  K

    AGGTTCGCCGGCTCACAGCCACATGAACAAGTCAATGAGTCGTTGGTGTGTTGTTTATCT
1141---------+---------+---------+---------+---------+---------+ 1200
    R  F  A  G  S  Q  P  H  E  Q  V  N  E  S  L  V  C  C  L  S

    TCATTCCACATATGGGACGCAGTTCCAGATTTTCATGTCAAATAATTGCGTCGTGTGCGG
1201---------+---------+---------+---------+---------+---------+ 1260
    S  F  H  I  W  D  A  V  P  D  F  H  V  K

    TTGTCAAGACTCAAATAGGAGAAAAAAAGACTCGTGATTTCGTTTTGCAAAAAAAAAAAA
1261---------+---------+---------+---------+---------+---------+ 1320

    AAAAA
1321 -----  1325
```

FIGURE 13 CONTINUED

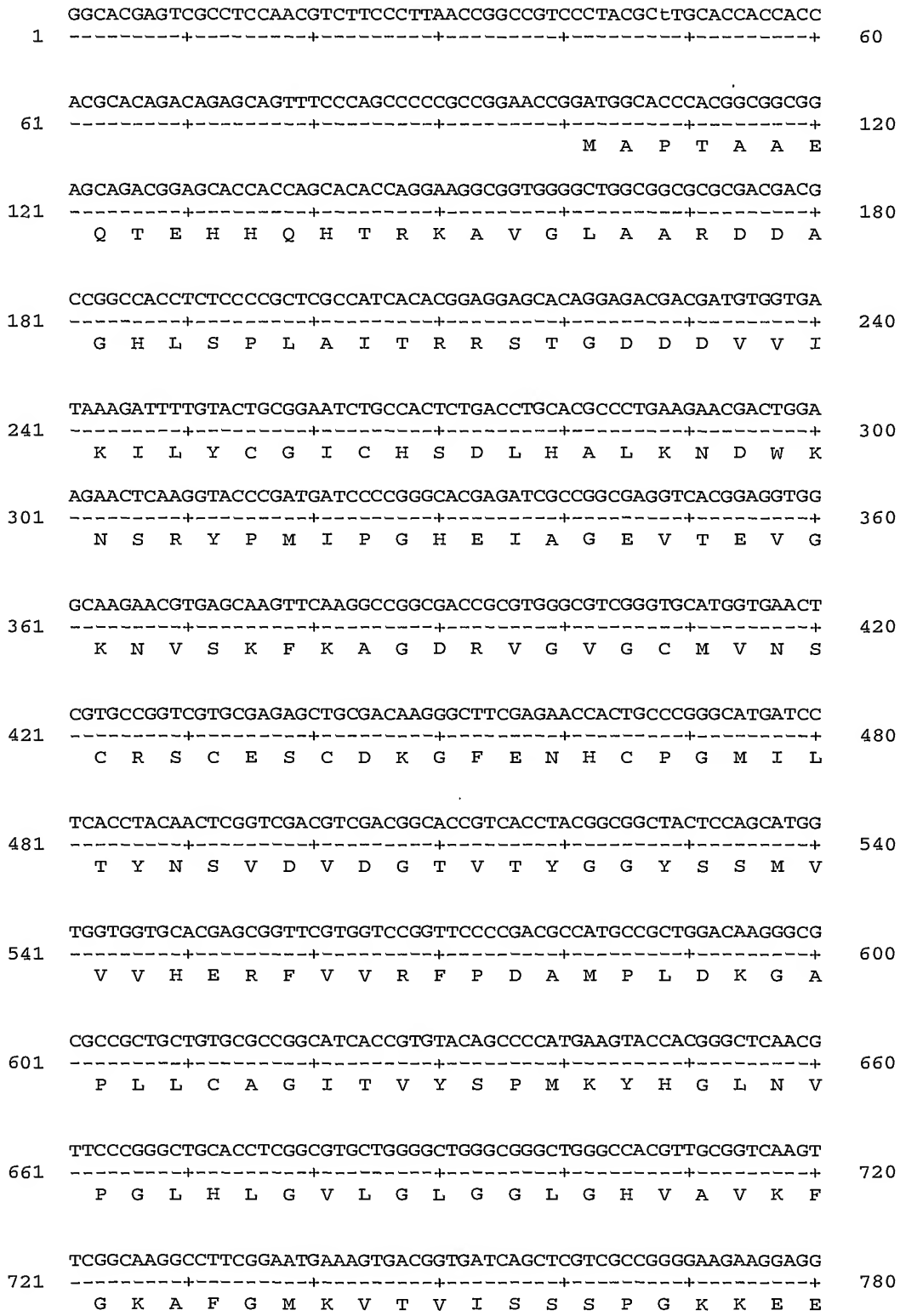

GGCACGAGTCGCCTCCAACGTCTTCCCTTAACCGGCCGTCCCTACGCtTGCACCACCACC
1 60
ACGCACAGACAGAGCAGTTTCCCAGCCCCCGCCGGAACCGGATGGCACCCACGGCGGCGG
61 120
M A P T A A E
AGCAGACGGAGCACCACCAGCACACCAGGAAGGCGGTGGGGCTGGCGGCGCGCGACGACG
121 180
Q T E H H Q H T R K A V G L A A R D D A
CCGGCCACCTCTCCCCGCTCGCCATCACACGGAGGAGCACAGGAGACGACGATGTGGTGA
181 240
G H L S P L A I T R R S T G D D D V V I
TAAAGATTTTGTACTGCGGAATCTGCCACTCTGACCTGCACGCCCTGAAGAACGACTGGA
241 300
K I L Y C G I C H S D L H A L K N D W K
AGAACTCAAGGTACCCGATGATCCCCGGGCACGAGATCGCCGGCGAGGTCACGGAGGTGG
301 360
N S R Y P M I P G H E I A G E V T E V G
GCAAGAACGTGAGCAAGTTCAAGGCCGGCGACCGCGTGGGCGTCGGGTGCATGGTGAACT
361 420
K N V S K F K A G D R V G V G C M V N S
CGTGCCGGTCGTGCGAGAGCTGCGACAAGGGCTTCGAGAACCACTGCCCGGGCATGATCC
421 480
C R S C E S C D K G F E N H C P G M I L
TCACCTACAACTCGGTCGACGTCGACGGCACCGTCACCTACGGCGGCTACTCCAGCATGG
481 540
T Y N S V D V D G T V T Y G G Y S S M V
TGGTGGTGCACGAGCGGTTCGTGGTCCGGTTCCCCGACGCCATGCCGCTGGACAAGGGCG
541 600
V V H E R F V V R F P D A M P L D K G A
CGCCGCTGCTGTGCGCCGGCATCACCGTGTACAGCCCCATGAAGTACCACGGGCTCAACG
601 660
P L L C A G I T V Y S P M K Y H G L N V
TTCCCGGGCTGCACCTCGGCGTGCTGGGGCTGGGCGGGCTGGGCCACGTTGCGGTCAAGT
661 720
P G L H L G V L G L G G L G H V A V K F
TCGGCAAGGCCTTCGGAATGAAAGTGACGGTGATCAGCTCGTCGCCGGGGAAGAAGGAGG
721 780
G K A F G M K V T V I S S S P G K K E E

FIGURE 14

```
                AGGCCCTGGGGCGGCTGGGCGCCGACGCGTTCATCGTCAGCAAGGACGCCGACGAGATGA
781             ----------+---------+---------+---------+---------+---------+  840
                  A  L  G  R  L  G  A  D  A  F  I  V  S  K  D  A  D  E  M  K

                AGGCTGTGATAGCACCATGGATGGCATCANTAAACACGGTATCTGCAAACATCCCCCTGA
841             ----------+---------+---------+---------+---------+---------+  900
                  A  V  I  A  P  W  M  A  S  X  N  T  V  S  A  N  I  P  L  T

                CCCCTCTCTTCGGGCTGCTCAAGCCCAACGGCAAGATGATCATGGTCGGCCTCCCCGAGA
901             ---------+---------+---------+---------+---------+---------+  960
                  P  L  F  G  L  L  K  P  N  G  K  M  I  M  V  G  L  P  E  K

                AGCCCATCGAGATTCCTCCCTTCGCTCTAGTTGCCACGAATAAGACCCTGGCCGGGAGCA
961             ---------+---------+---------+---------+---------+---------+ 1020
                  P  I  E  I  P  P  F  A  L  V  A  T  N  K  T  L  A  G  S  I

                TCATCGGCGGCATGAGCGACACGCAGGAGATGCTGGACCTCGCGGCGAAGCACGGCGTGA
1021            ---------+---------+---------+---------+---------+---------+ 1080
                  I  G  G  M  S  D  T  Q  E  M  L  D  L  A  A  K  H  G  V  T

                CGGCCGACATCGAGGTGGTCGGCGCGGAGTATGTGAACACGGCCTTGGAGCGCCTTGCCA
1081            ---------+---------+---------+---------+---------+---------+ 1140
                  A  D  I  E  V  V  G  A  E  Y  V  N  T  A  L  E  R  L  A  K

                AGAACGACGTCAGGTATCGCTTCGTCATCGACATCGGCAACACCCTCGACAATGTTGCGG
1141            ---------+---------+---------+---------+---------+---------+ 1200
                  N  D  V  R  Y  R  F  V  I  D  I  G  N  T  L  D  N  V  A  A

                CCACCACCGAGTGAACGTACTCAGCACTGCTTACGATCTACGTTGTTCCACTGTTAGTGC
1201            ---------+---------+---------+---------+---------+---------+ 1260
                  T  T  E  *

                TCCGTAGTAAACAATAAACGATCAAAACTCTTGTCATCTGGTGCATTGGTGTAGACATGG
1261            ---------+---------+---------+---------+---------+---------+ 1320

                TTGTTTGCGAGGAAACTGAGTTGAAGGATGGATGGATAAAAAAAAAAAAAAAAAAAAA
1321            ---------+---------+---------+---------+---------+-------- 1378
```

FIGURE 14 CONTINUED

FIGURE 15

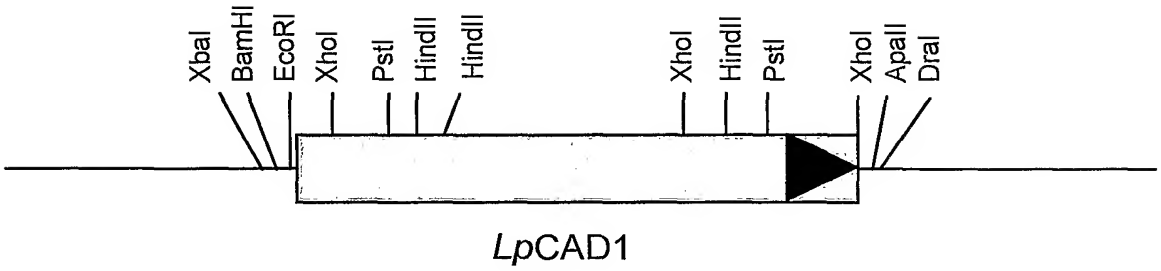


FIGURE 16

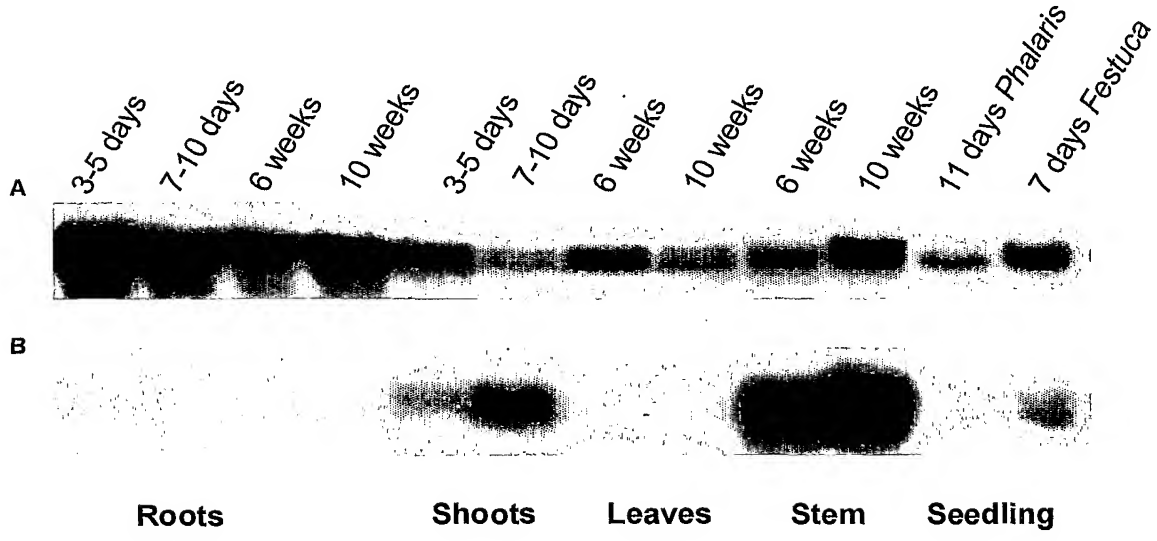
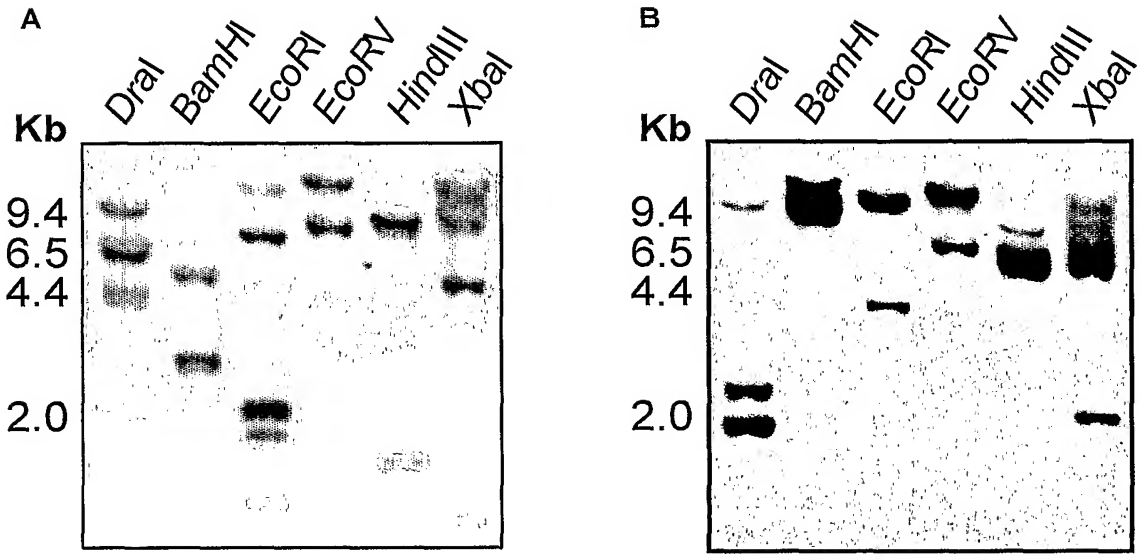


FIGURE 17


A
Kb
DraI
BamHI
EcoRI
EcoRV
HindIII
XbaI
9.4
6.5
4.4
2.0
B
Kb
DraI
BamHI
EcoRI
EcoRV
HindIII
XbaI
9.4
6.5
4.4
2.0

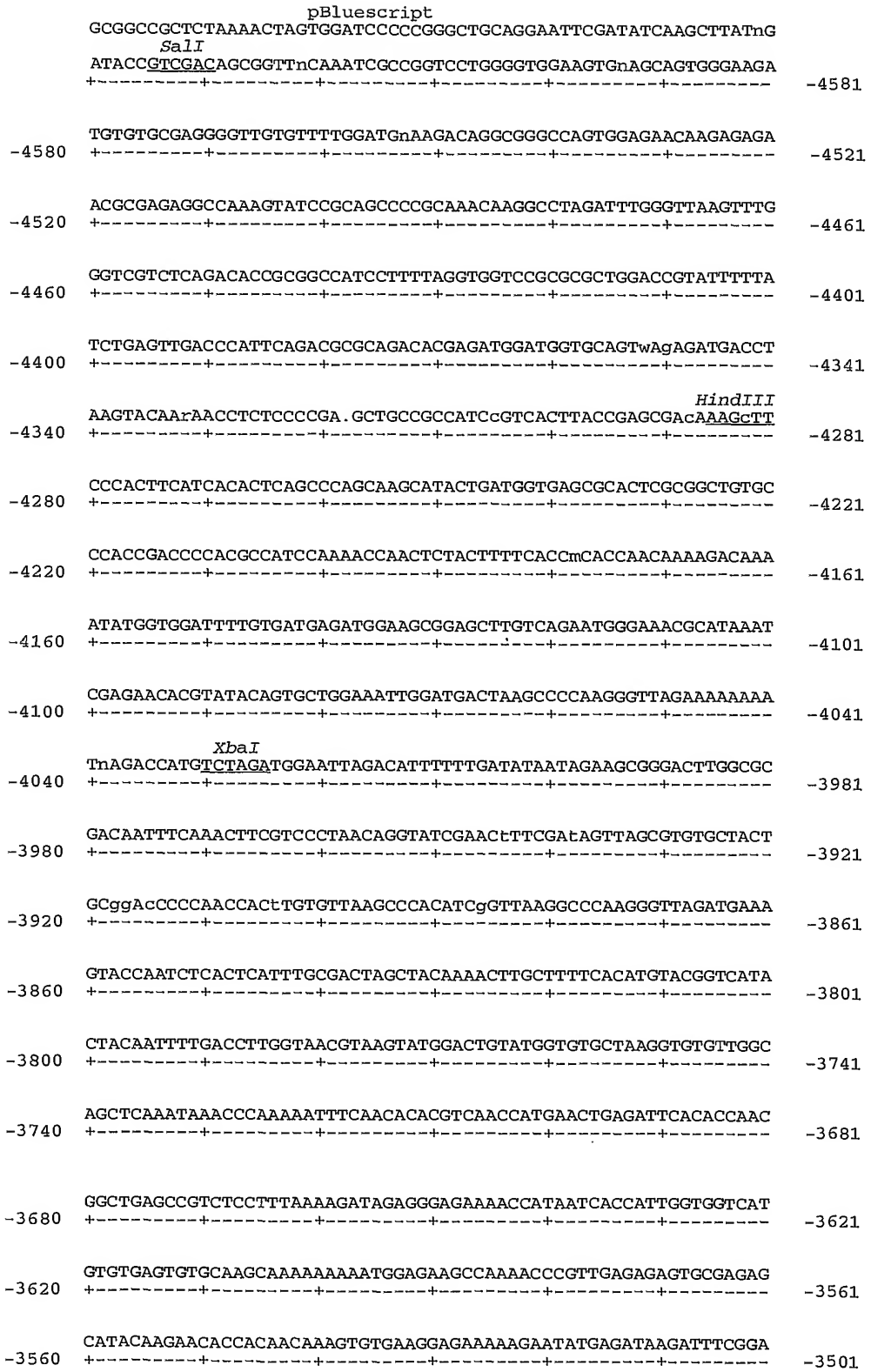

pBluescript
GCGGCCGCTCTAAAACTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTATnG
SalI
ATACCGTCGACAGCGGTTnCAAATCGCCGGTCCTGGGGTGGAAGTGnAGCAGTGGGAAGA
-4581
TGTGTGCGAGGGGTTGTGTTTTGGATGnAAGACAGGCGGGCCAGTGGAGAACAAGAGAGA
-4580
-4521
ACGCGAGAGGCCAAAGTATCCGCAGCCCCGCAAACAAGGCCTAGATTTGGGTTAAGTTTG
-4520
-4461
GGTCGTCTCAGACACCGCGGCCATCCTTTTAGGTGGTCCGCGCGCTGGACCGTATTTTTA
-4460
-4401
TCTGAGTTGACCCATTCAGACGCGCAGACACGAGATGGATGGTGCAGTwAgAGATGACCT
-4400
-4341
HindIII
AAGTACAArAACCTCTCCCCGA.GCTGCCGCCATCcGTCACTTACCGAGCGAcAAAGcTT
-4340
-4281
CCCACTTCATCACACTCAGCCCAGCAAGCATACTGATGGTGAGCGCACTCGCGGCTGTGC
-4280
-4221
CCACCGACCCCACGCCATCCAAAACCAACTCTACTTTTCACCmCACCAACAAAAGACAAA
-4220
-4161
ATATGGTGGATTTTGTGATGAGATGGAAGCGGAGCTTGTCAGAATGGGAAACGCATAAAT
-4160
-4101
CGAGAACACGTATACAGTGCTGGAAATTGGATGACTAAGCCCCAAGGGTTAGAAAAAAAA
-4100
-4041
XbaI
TnAGACCATGTCTAGATGGAATTAGACATTTTTTGATATAATAGAAGCGGGACTTGGCGC
-4040
-3981
GACAATTTCAAACTTCGTCCCTAACAGGTATCGAACtTTCGAtAGTTAGCGTGTGCTACT
-3980
-3921
GCggAcCCCCAACCACtTGTGTTAAGCCCACATCgGTTAAGGCCCAAGGGTTAGATGAAA
-3920
-3861
GTACCAATCTCACTCATTTGCGACTAGCTACAAAACTTGCTTTTCACATGTACGGTCATA
-3860
-3801
CTACAATTTTGACCTTGGTAACGTAAGTATGGACTGTATGGTGTGCTAAGGTGTGTTGGC
-3800
-3741
AGCTCAAATAAACCCAAAAATTTCAACACACGTCAACCATGAACTGAGATTCACACCAAC
-3740
-3681
GGCTGAGCCGTCTCCTTTAAAAGATAGAGGGAGAAAACCATAATCACCATTGGTGGTCAT
-3680
-3621
GTGTGAGTGTGCAAGCAAAAAAAAATGGAGAAGCCAAAACCCGTTGAGAGAGTGCGAGAG
-3620
-3561
CATACAAGAACACCACAACAAAGTGTGAAGGAGAAAAAGAATATGAGATAAGATTTCGGA
-3560
-3501

FIGURE 18

```
                AATACTTTTGCACACCCATGCATGGGTGTGGGTGTTTCCGTCACCGTCTATGTATTTCTC
-3500           +---------+---------+---------+---------+---------+---------  -3441
                GAAATTCATGCCCACCATGGTAGATAAAAATATTTTTTTCTCTCTCCTCTTTTTATTCAA
-3440           +---------+---------+---------+---------+---------+---------  -3381
                ATCTCAAAGCAtAAkrArTGGTGACAGAACGATAAGATTCCTACCTAGCTTTCTGAGATC
-3380           +---------+---------+---------+---------+---------+---------  -3321
                CCACTAGTTTATCTTCAAGCTGGTGATTGAAGGATTAACCATGCTTGAATTAGATTGGCT
-3320           +---------+---------+---------+---------+---------+---------  -3261
                TCAAACTTGGTAGTAGCTTGTTTCATACTTTGATTACTTTGGTATGGTTAGTTGGTTTGA
-3260           +---------+---------+---------+---------+---------+---------  -3201
                GATTTTGGTCAATGTAGAATCAGATTTGAGAGCGATTGTCAGCTTGAATTGCCGCAGTTT
-3200           +---------+---------+---------+---------+---------+---------  -3141
                TAGCACATACTAGTTTGGATAGATGAACAGTTTGGAGAGACAAATAATGTCTATACGAGC
-3140           +---------+---------+---------+---------+---------+---------  -3081
                TCATCGGATAATATTAGTCTATGGCTTTTGCTTCGGTGTCCCCTCTGCAAACTTTACCCC
-3080           +---------+---------+---------+---------+---------+---------  -3021
                TCTGTAGATGGTAGGATTTTCTGATATCCTTTCATGGTTTAAGGGTGTGCGTGTAAGGAA
-3020           +---------+---------+---------+---------+---------+---------  -2961
                CGGGAGATACCGGATCACACCTTTTCGTCTACACTTTACAAGCATGTAACACCTAAGATT
-2960           +---------+---------+---------+---------+---------+---------  -2901
                GATTGATATCTAGGCTTACACCCCAATGGAGGTAAACTAATATTATTGAAATGCGACTTT
-2900           +---------+---------+---------+---------+---------+---------  -2841
                TCAAAAGTCCCAATATAACCTTGACGATGATCTTACAACTACTCGCGCCAGTCTTGTATG
-2840           +---------+---------+---------+---------+---------+---------  -2781
                                  KpnI
                ATATCAGATTGGCCGAGGATCGTGGGTACCTTGTAGTGGACTATGATGCTCATGGAGGTT
-2780           +---------+---------+---------+---------+---------+---------  -2721
                GTATGGACATGTTGTAATGCTGGTTTTCTCTAGGTTTTTTCTAATCAACTTGGCATTCTT
-2720           +---------+---------+---------+---------+---------+---------  -2661
                CTCCTTAACACATAATAAGAGGGAATACCTCCATACATTATTCTGAAAAAAGCATGGCCA
-2660           +---------+---------+---------+---------+---------+---------  -2601
                ACAATGAAACAGAAACAAGTACGACAGTCTATACCCGACCCAAACAATGGCTCAGGTCTT
-2600           +---------+---------+---------+---------+---------+---------  -2541
                TCACGATGCATAGTTTGTTAGCATGTATTTTATAGTAGGAACTAAAATTTAAAGACAACT
-2540           +---------+---------+---------+---------+---------+---------  -2481
                TGCnAAAACAATTTTGTCTCTTGAGTGTTTTTTAAGGATGCGGCATTTATCGATTATACA
-2480           +---------+---------+---------+---------+---------+---------  -2421
                TTACATATGTGATTGGATtAGCCAACTTTTTGTCTTCCgATGATCATATGAAAGGGTTGT
-2420           +---------+---------+---------+---------+---------+---------  -2361
                ATCTTAGGGCATCTCCAATGGGnAGACTCAAATGCAAAAAAATnGTCCGTTTGGGTCTTC
-2360           +---------+---------+---------+---------+---------+---------  -2301
                CnGGACAAAACCTGCTCCCAACGGGGCAACCCAACTTAAAAACGGACAGGTGCAGCGTCC
-2300           +---------+---------+---------+---------+---------+---------  -2241
```

FIGURE 18 CONTINUED

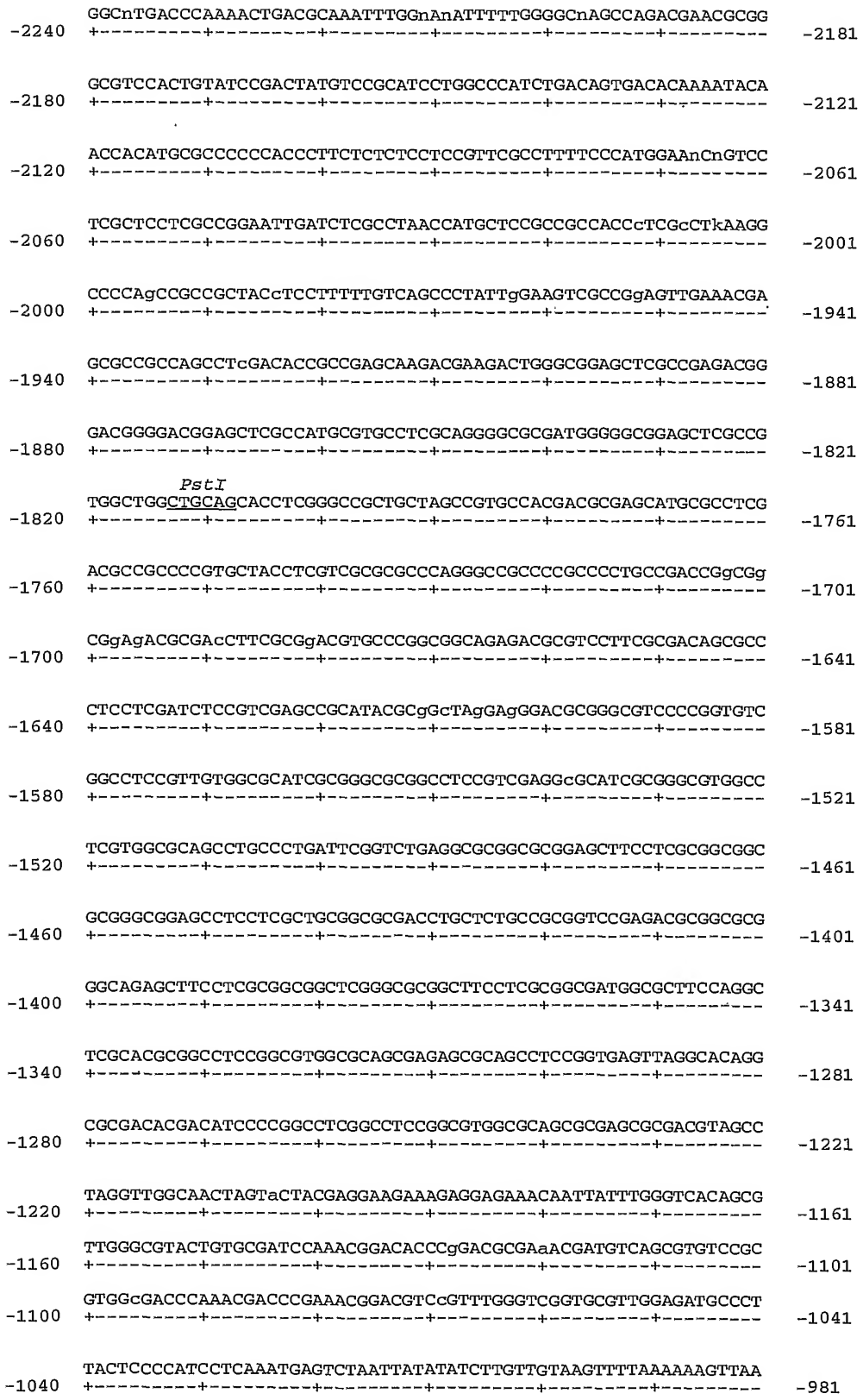


FIGURE 18 CONTINUED

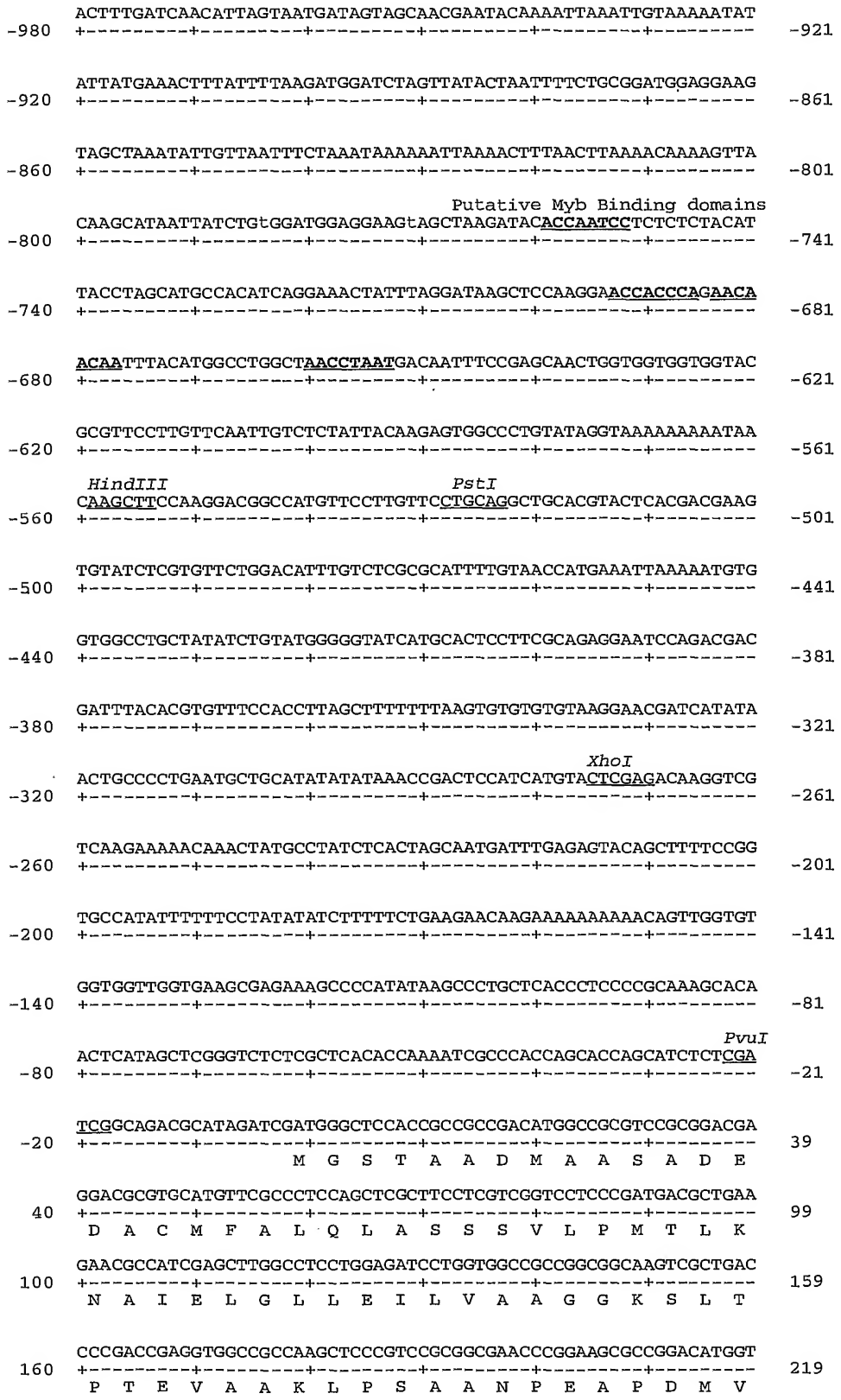


FIGURE 18 CONTINUED

```
     GGACCGCATACTCCGGCTGCTCGCGTCGTACAACGTCGTGACGTGCCTGGTGGAGGAGGG
220  +---------+---------+---------+---------+---------+---------  279
      D  R  I  L  R  L  L  A  S  Y  N  V  V  T  C  L  V  E  E  G

     CAAGGACGGCCGCCTCTCCCGGAGCTACGGCGCCGCGCCCGTGTGCAAGTTCCTCACCCC
280  +---------+---------+---------+---------+---------+---------  339
      K  D  G  R  L  S  R  S  Y  G  A  A  P  V  C  K  F  L  T  P

     CAACGAGGACGGCGTCTCCATGGCGGCGCTCGCGCTCATGAACCAGGACAAGGTCCTCAT
340  +---------+---------+---------+---------+---------+---------  399
      N  E  D  G  V  S  M  A  A  L  A  L  M  N  Q  D  K  V  L  M

           Intron/exon boundary
     GGAGAGCTG↓GTGAGTCTCTCAGTGGAGCTAGTTACTGTAGATCCGAATTCGTTCCCTTTA
400  +---------+---------+---------+---------+---------+---------  459
      E  S
                                  SalI          pBluescript
     GTGAGGGTTAATTCCGCGGCCGCGTCGACCTCGAGGGGGGGCCCGGTACCCAATTCGCCC
460  +---------+---------+---------+

     TATAGTGAGTCGTATTACGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAA
     AACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGT
     AATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
     TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTTGTG   744
```

FIGURE 18 CONTINUED

FIGURE 19

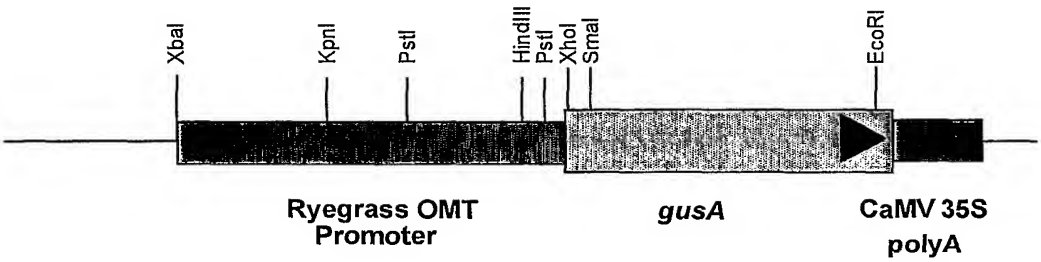

XbaI
KpnI
PstI
HindIII
PstI
XhoI
SmaI
EcoRI
Ryegrass OMT Promoter
gusA
CaMV 35S polyA

FIGURE 20

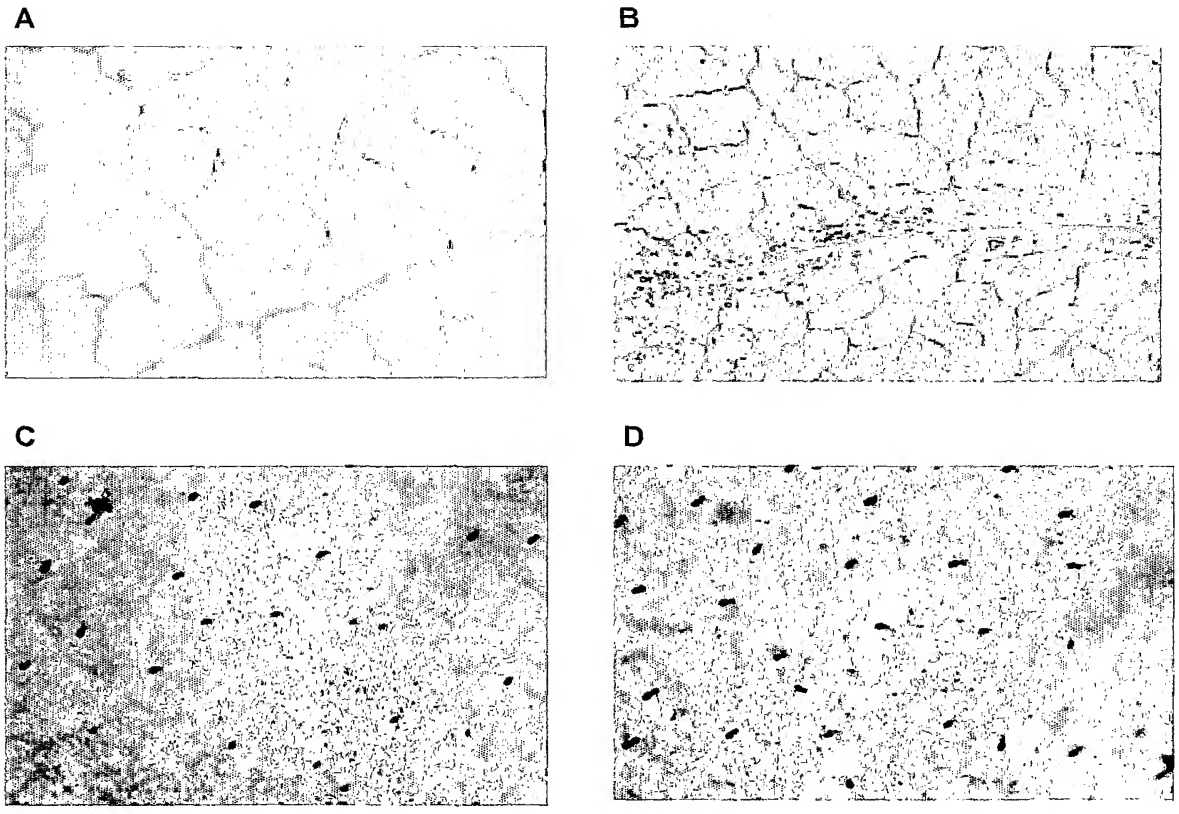
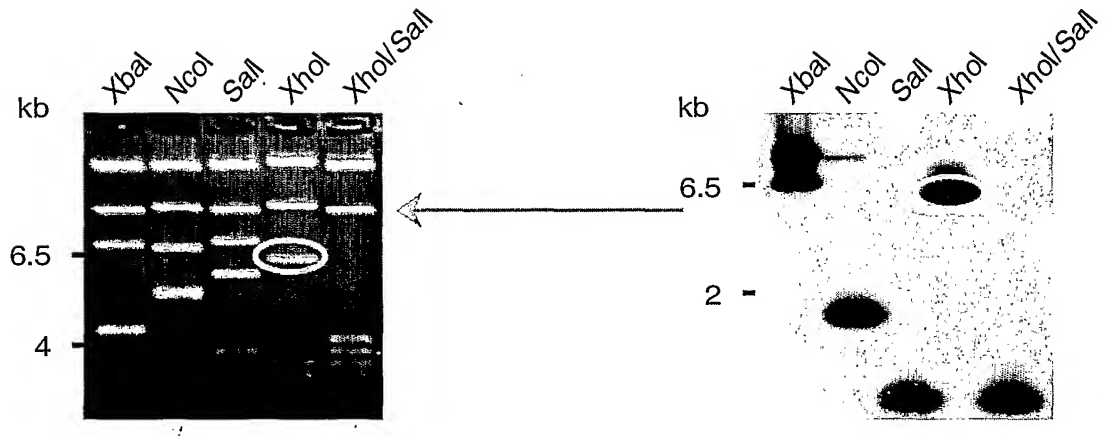
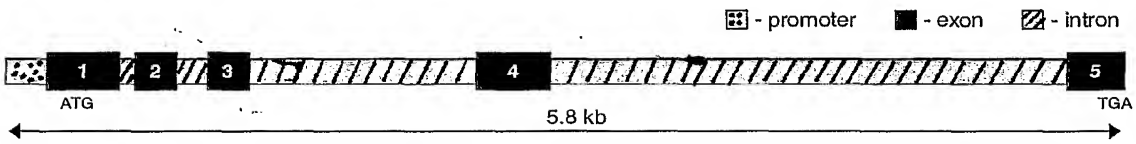


FIGURE 21

A



B

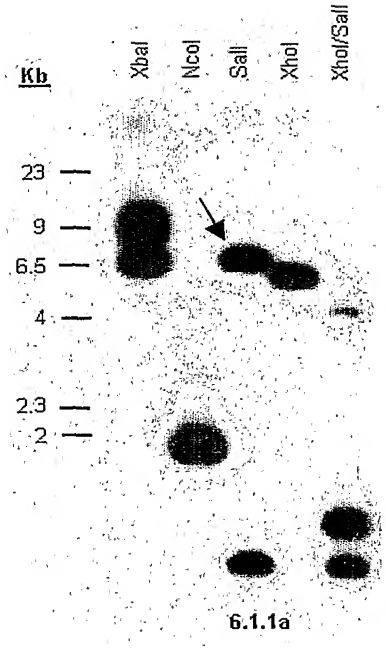


C

Exon	*Lp*CCR1	*Eg*CCR1	*Es*CCR1	*Pb*CCR1
1	173 bp	133 bp	133 bp	139 bp
2	155 bp	155 bp	155 bp	155 bp
3	189 bp	186 bp	186 bp	186 bp
4	353 bp	353 bp	353 bp	353 bp
5	220 bp	218 bp	184 bp	184 bp

FIGURE 22

A


Kb
XbaI
NcoI
SalI
XhoI
XhoI/SalI
23
9
6.5
4
2.3
2
6.1.1a

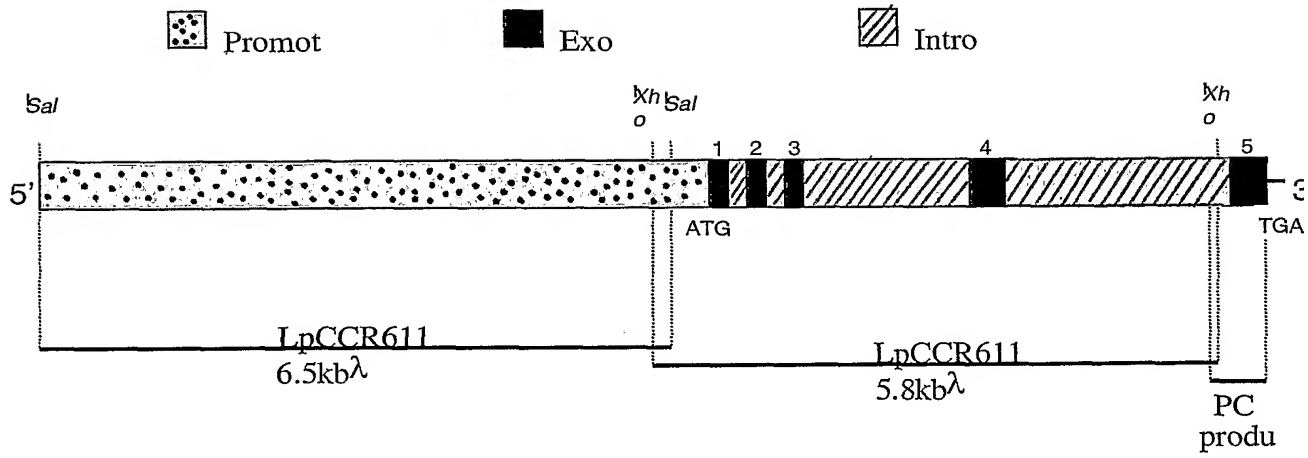

Promot
Exo
Intro
Sal
Xho
Sal
Xho
1
2
3
4
5
5'
3
ATG
TGA
LpCCR611
6.5kbλ
LpCCR611
5.8kbλ
PC
produ

FIGURE 23

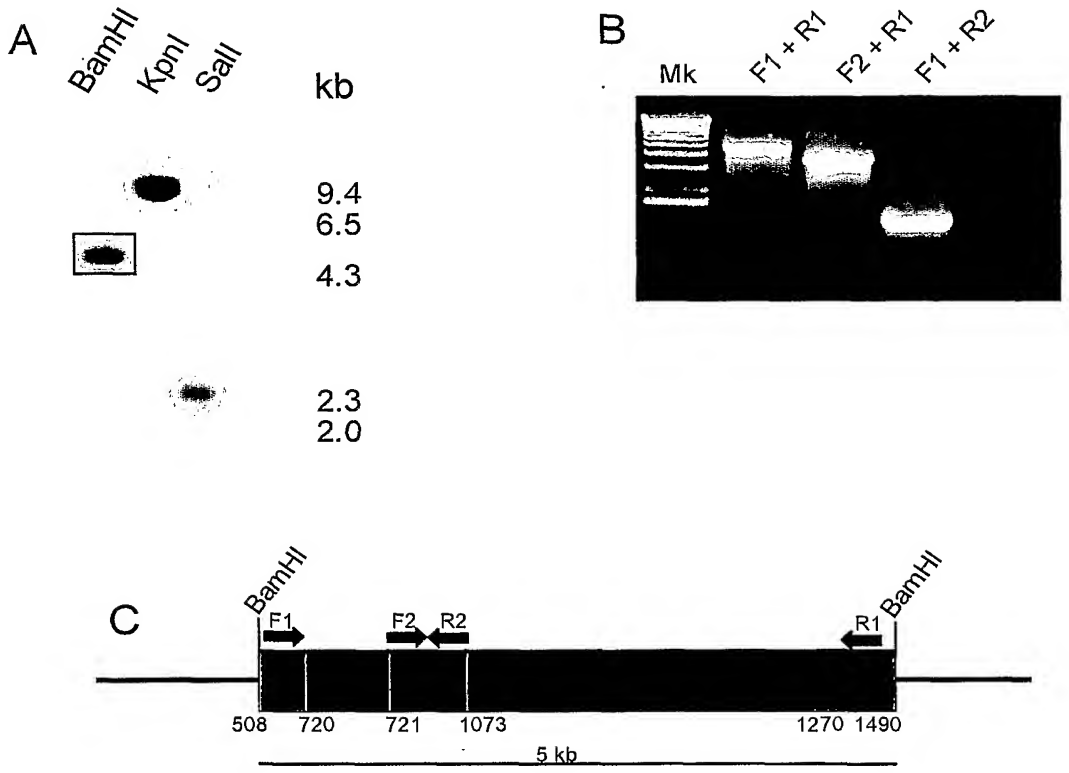

A
BamHI
KpnI
SalI
kb
9.4
6.5
4.3
2.3
2.0
B
Mk
F1 + R1
F2 + R1
F1 + R2
C
BamHI
F1
F2
R2
R1
BamHI
508
720
721
1073
1270
1490
5 kb

FIGURE 24

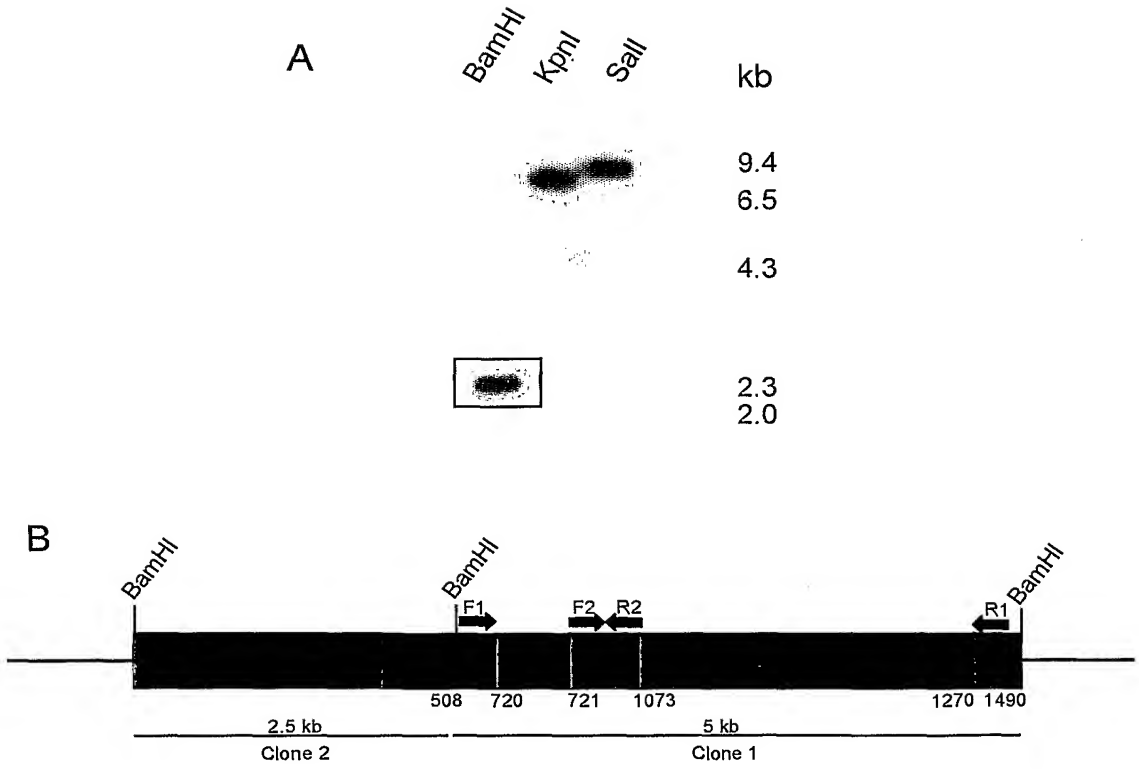
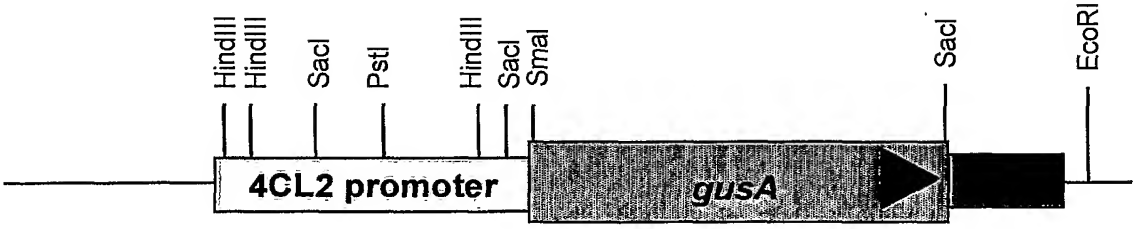

A
BamHI
KpnI
SalI
kb
9.4
6.5
4.3
2.3
2.0
B
BamHI
BamHI
F1
F2
R2
R1
BamHI
508
720
721
1073
1270
1490
2.5 kb
5 kb
Clone 2
Clone 1

FIGURE 25


HindIII
HindIII
SacI
PstI
HindIII
SacI
SmaI
SacI
EcoRI
4CL2 promoter
gusA

```
    TCCCGTATCTTCAACGTGACACCCCTACACTTCCTGCTTGTCTTGGAGATTTACACACAC
  1 ---------+---------+---------+---------+---------+---------+   60

    ACGGCAATTACCAGGAGTATCTTCCTAGATTATTTTTTTCGATAAGGATCTTCCAGATAT
 61 ---------+---------+---------+---------+---------+---------+  120

    AGCATGTGAATCTCTGTACTACTACTGTTTGTCAAGCAAAATTAACATTGACATCAGTGT
121 ---------+---------+---------+---------+---------+---------+  180

    TTTTGTTGGGGGCAGCGGAATCTTTGACGCCTCTTCTTGCCTCTCAAGACATGTCACCCT
181 ---------+---------+---------+---------+---------+---------+  240

    CACTAGTTAGTGTGCCAGCTGGTAGTACTACGTACGATGCTCCCTCCCTCCGTAATTATT
241 ---------+---------+---------+---------+---------+---------+  300

    CAACCTTTTTGCTCTCTCTTTTTATAAAGTCAAACCTTTTAAATCTGACCAGATATCTGC
301 ---------+---------+---------+---------+---------+---------+  360

    TAAAAAATTAGCAGACATGCATACATCAAAGCAGTAGTCCTCCCTCCGTTTAAAATTACC
361 ---------+---------+---------+---------+---------+---------+  420

    TGGGTTTATTCAAATAAAGTCAAACTCTGTAAAATTCAATTAAATATTTAGAAAAATCTA
421 ---------+---------+---------+---------+---------+---------+  480

    ACAGCACCTGTAGTATAAAAGTATGCTCCCTCTGTTTGTAAAAAAGCTAAGCAACTTTTT
481 ---------+---------+---------+---------+---------+---------+  540

    TGAGATACGGATAAATCTTTAGCTAAAACATGTCTATATACCTTTGTATCTAGATAAAGT
541 ---------+---------+---------+---------+---------+---------+  600

    TGGAAAGCTTTTTTAGAAACAGACAAAGTATGTGTTTGACATTATGAATGTTGAGTATTT
601 ---------+---------+---------+---------+---------+---------+  660

    TTCCTCTAATCTTGATCAAATTTTACAAATTTTGGCTTGAATAGAGGGACCATTATTAGT
661 ---------+---------+---------+---------+---------+---------+  720

    ATGAAACTACATAAATTTGTAAAACACTCAACATAATTTACGATGGGTCAGTGATAGCAC
721 ---------+---------+---------+---------+---------+---------+  780

    TAACTTAGCTTTTCATAAATGCCACTGCTTTTCAATAGAGCATGAAGCAGGACAAATTTA
781 ---------+---------+---------+---------+---------+---------+  840

    TTCGTGTGACTTGAATAGAGGGAGCCTGTTCTGGTTCAACTCACCCTGCATGTGTGTCTT
841 ---------+---------+---------+---------+---------+---------+  900

    CATCCCTTTTGCTCTTCCTATCTGTGGTGTCAATTGAGTGTCCCACGTGCATGTGGGCGA
901 ---------+---------+---------+---------+---------+---------+  960
```

FIGURE 26

```
      AACTTGAACCTAGAAATTGACATGCTCCCACTGCCCGGAGCGGAGTATCTTTGTGCTTTG
 961  ---------+---------+---------+---------+---------+---------+  1020

      TTACCCTTATTGTTGCTACGTACTACAGTGTTTAGATTGGAACTTCATAATCAAAAGAAC
1021  ---------+---------+---------+---------+---------+---------+  1080

      TTAGTTTCCTACAATTTTTTGCTAAGCAATATAATGAGCAATCAAACTTCTATATCTGTG
1081  ---------+---------+---------+---------+---------+---------+  1140

      GCAAATAACTAATCCATTATAGTTACAGTTTAGATGCAGACGCCAGTGTTTCTTCCCCTT
1141  ---------+---------+---------+---------+---------+---------+  1200

      TTCGGAAAAAAGCTATTCCATAATAAGTGTTGGAAATTTAATAAATGGGTACTACGAATT
1201  ---------+---------+---------+---------+---------+---------+  1260

      TGAAAAAAAAAGTGTCAAAAATTCACTAAGAAAGTACGTAGTACAAATTTAAACTAAGAT
1261  ---------+---------+---------+---------+---------+---------+  1320

      TCCGACACTTATTAGGATCGGAGAGAGTAAGTAGCAAACTACTACTCCATCCACCTAAAA
1321  ---------+---------+---------+---------+---------+---------+  1380

      CACGTGATTTAACTTTGTCTAGATACGGATAGAAAGTTGGGATACATCCGTATCTTAAAA
1381  ---------+---------+---------+---------+---------+---------+  1440

      AAAAACGCACTTATTTAGACGAAGGAGGGAGTATTTCAACCTTGATTTTAAACGGAATC
1441  ---------+---------+---------+---------+---------+---------+  1500

      TACAAAGGGAATACATGGATTGTACAAGTGGGCTGACCGTATCCATTATGTACTCGTACT
1501  ---------+---------+---------+---------+---------+---------+  1560

      TTGCAGTTTGAAAGCAAAGGCTAGTGTAATTTGTAGGTGGTTCTAGGCGTCTAGCTGTTT
1561  ---------+---------+---------+---------+---------+---------+  1620

      CATGGCGTTATCACAGCCGTGCCAGTGTGCTCAGGGCCGTACATAAGTTGCTTGGTGTAT
1621  ---------+---------+---------+---------+---------+---------+  1680

      GTGTCGATCTAGGATTTGCCGTCTTACAATTTTGCTTTCCAACTTATTTTCTGTAAAGAG
1681  ---------+---------+---------+---------+---------+---------+  1740

      ATCGATGTGAACTTCTCTGTCGAGTAAACTGAAATTGTCTGAATAAATATAACTCGGCAG
1741  ---------+---------+---------+---------+---------+---------+  1800

      ATTATGTTTTATCGTTTGCATGCGTAACAGGCTACACAAATTGCTCGAGTCAGCAGCGAG
1801  ---------+---------+---------+---------+---------+---------+  1860

      TTGAGCTCACAACGAATCCATCAGCAAAAATACTATACTATAGTAGCACATCGTTTCTTT
1861  ---------+---------+---------+---------+---------+---------+  1920
```

FIGURE 26 CONTINUED

```
                TTTCATGACGTTTCTGTTTCTTCCTAACTTTCCAGGAGCACCGGAGACGACGATGTGGTG
1921            ---------+---------+---------+---------+---------+---------+  1980
                                                R  S  T  G  D  D  D  V  V

                ATAAAGATTTTGTACTGCGGAATCTGCCACTCTGACCTGCACGCCCTGAAGAACGACTGG
1981            ---------+---------+---------+---------+---------+---------+  2040
                I  K  I  L  Y  C  G  I  C  H  S  D  L  H  A  L  K  N  D  W

                AAGAACTCAAGGTACCCGATGATCCCCGGGCACGAGATCGCCGGCGAGGTCACGGAGGTG
2041            ---------+---------+---------+---------+---------+---------+  2100
                K  N  S  R  Y  P  M  I  P  G  H  E  I  A  G  E  V  T  E  V

                GGCAAGAACGTGAGCAAGTTCAAGGCCGGCGACCGCGTGGGCGTCGGGTGCATGGTGAAC
2101            ---------+---------+---------+---------+---------+---------+  2160
                G  K  N  V  S  K  F  K  A  G  D  R  V  G  V  G  C  M  V  N

                TCGTGCCGGTCGTGCGAGAGCTGCGACAAGGGCTTCGAGAACCACTGCCCGGGCATGATC
2161            ---------+---------+---------+---------+---------+---------+  2220
                S  C  R  S  C  E  S  C  D  K  G  F  E  N  H  C  P  G  M  I

                CTCACCTACAACTCGGTCGACGTCGACGGCACCGTCACCTACGGCGGCTACTCCAGCATG
2221            ---------+---------+---------+---------+---------+---------+  2280
                L  T  Y  N  S  V  D  V  D  G  T  V  T  Y  G  G  Y  S  S  M

                GTGGTGGTGCACGAGCGGTTCGTGGTCCGGTTCCCCGACGCCATGCCGCTGGACAAGGGC
2281            ---------+---------+---------+---------+---------+---------+  2340
                V  V  V  H  E  R  F  V  V  R  F  P  D  A  M  P  L  D  K  G

                GCGCCGCTGCTGTGCGCCGGCATCACCGTGTACAGCCCCATGAAGTACCACGGGCTCAAC
2341            ---------+---------+---------+---------+---------+---------+  2400
                A  P  L  L  C  A  G  I  T  V  Y  S  P  M  K  Y  H  G  L  N

                GTTCCCGGGCTGCACCTCGGCGTGCTGGGGCTGGGCGGGCTGGGCCACGTTGCGGTCAAG
2401            ---------+---------+---------+---------+---------+---------+  2460
                V  P  G  L  H  L  G  V  L  G  L  G  G  L  G  H  V  A  V  K

                TTCGGCAAGGCCTTCGGAATGAAAGTGACGGTGATCAGCTCGTCGCCGGGGAAGAAGGAG
2461            ---------+---------+---------+---------+---------+---------+  2520
                F  G  K  A  F  G  M  K  V  T  V  I  S  S  S  P  G  K  K  E

                GAGGCCCTGGGGCGGCTGGGCGCCGACGCGTTCATCGTCAGCAAGGACGCCGACGAGATG
2521            ---------+---------+---------+---------+---------+---------+  2580
                E  A  L  G  R  L  G  A  D  A  F  I  V  S  K  D  A  D  E  M

                AAGGTAGGCGGACCCGCTGGTTCAGGTTACTTCCCCTGTCCGGTGCAGAAGAAAGAGGAA
2581            ---------+---------+---------+---------+---------+---------+  2640

                K
```

FIGURE 26 CONTINUED

G at 851 bp (coding sequence) missing from cDNA in cv Ellett

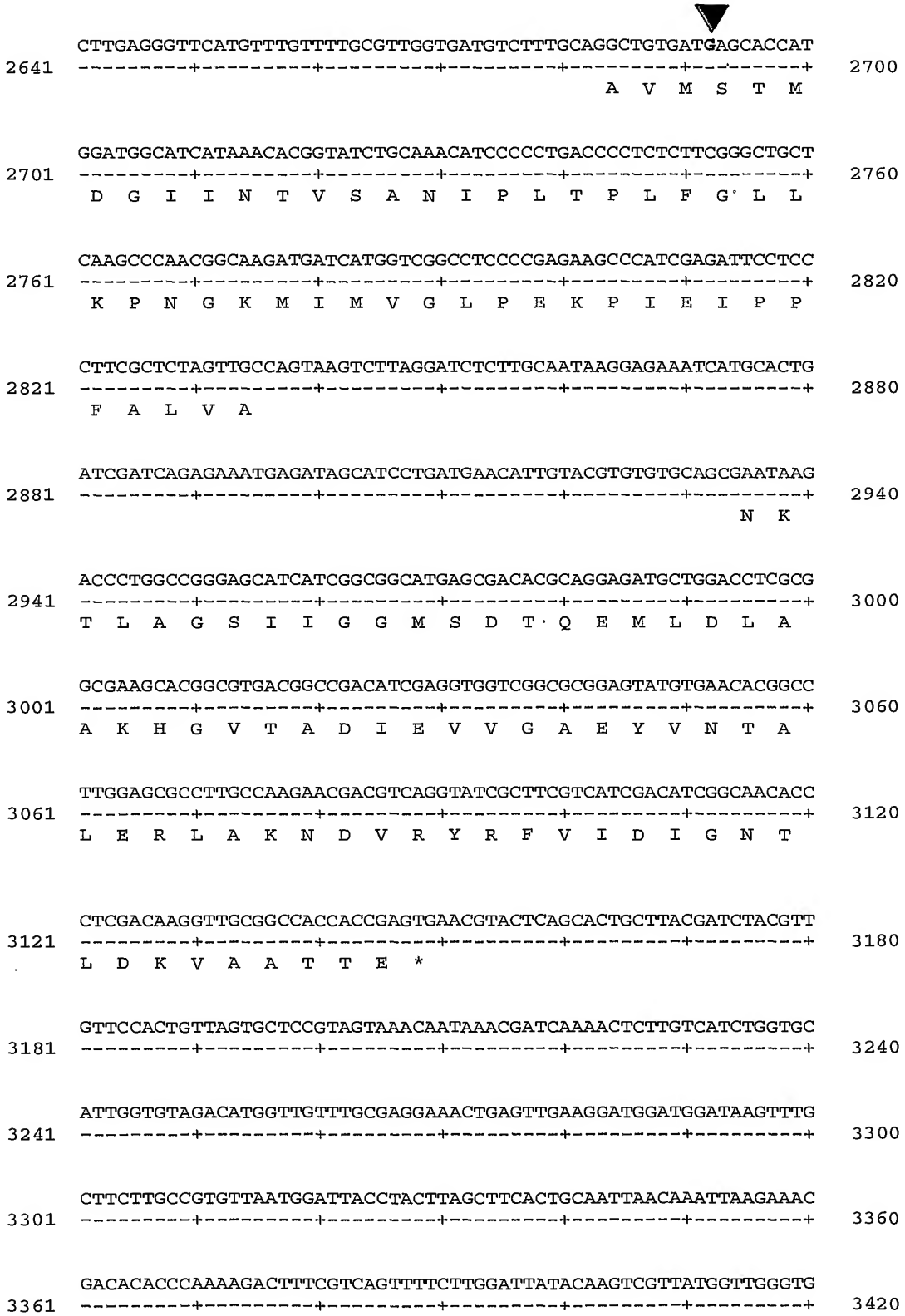


FIGURE 26 CONTINUED

```
                                                                    TCAGTGTGTCACAGATAATCATACTATGGTATTTAACCTGGAAGATCGTTTTTTTGGCGG
3421 ---------+---------+---------+---------+---------+---------+ 3480

CAACTCAGTGGGTTTTCCCACTATGTATATTTATAAATATTCAACAAGTCATGAGGTACA
3481 ---------+---------+---------+---------+---------+---------+ 3540

AAGGGTTGTTGCTAGAGGATAGCAACAAGAAGCTAGCCAAAAGATCATAGGCTTAAAAAA
3541 ---------+---------+---------+---------+---------+---------+ 3600

GAGAGAAAAGAAAACAAAACTGCTATAGTTATCGAAATCTCTCAGCTCAAATTTTAAAAC
3601 ---------+---------+---------+---------+---------+---------+ 3660

CAGCATAAGACTTTCTAGAAGCCTTATGAACAAGAAGAGCTAGCTCATCTTTAAACCTTT
3661 ---------+---------+---------+---------+---------+---------+ 3720

TCCTGCATCTGTAAAGATTGAGGGTGCAACCCTTGAATATAAAATCATTCCTGTCATCCA
3721 ---------+---------+---------+---------+---------+---------+ 3780

GATAGACTATGTAGTCAAAATAGTCATTTCCATGAAGAAGGGCACTTTTAATACATTTTT
3781 ---------+---------+---------+---------+---------+---------+ 3840

GAGACTTGGTATGATACTCTGAATGTCAACACCCTGGAAGATCTTTTCACTCCTATGGAA
3841 ---------+---------+---------+---------+---------+---------+ 3900

GGACAAGAAAGCATTTCAACTCCTTTTACTAAGGAAGAGATTGACAAGGTGATTCAGAGA
3901 ---------+---------+---------+---------+---------+---------+ 3960

ATTCCTTTAGACACTATAGAAAGTCACAAGGTGCCAACGGCGCAATCCTGTGCCGACGGC
3961 ---------+---------+---------+---------+---------+---------+ 4020

TTTTTATCGGGGAAGCCAGCATCGGTACCGAGACCGGCAGCCCACCAACTAGGCCGTCGG
4021 ---------+---------+---------+---------+---------+---------+ 4080

CACACATCCTCCAGTGTCGGCGGCCAACATCGGCATAAGTTGGCCCGTTGGGCATCAACT
4081 ---------+---------+---------+---------+---------+---------+ 4140

CCCCCGTCGGAACAGGTCTAGCGCATGGACCGTCGTGATGGCGGCGGCAACGACGTCATC
4141 ---------+---------+---------+---------+---------+---------+ 4200

CTATGCCGACGGCCTAGCCGTCGGCCTAGCTTGCCAGCGCTATGCCGACGTCACATTGCC
4201 ---------+---------+---------+---------+---------+---------+ 4260

ATCGGCACATGCTAGTTTTTTTTTCTTTTTTCTACATGCCAAATTGTATATGTATATATA
4261 ---------+---------+---------+---------+---------+---------+ 4320

CTCATTTACTTATTACTTCCAATTATTTTAATGTGTATATATTTTGCTCACCAATTGTAC
4321 ---------+---------+---------+---------+---------+---------+ 4380
```

FIGURE 26 CONTINUED

```
      GAATTTGTACCCTCCGAGAAATTGCTAAAATGATGGAGTGACCTACAACGAGCCTTGGAT
4381  ---------+---------+---------+---------+---------+---------+  4440

      ATGTGAGTTCTTCTTGCCCCATTGCACAAAAATTGTAAATATTAGGGTTTACTGGATCCA
4441  ---------+---------+---------+---------+---------+---------+  4500

      CTAGTTCTAGAGCGGCCGCCACCGCGGGGAGCTCCAGCTTTTGTTCCCTTTAGTA
A)    ---------+---------+---------+---------+---------+-----  4555
```

FIGURE 26 CONTINUED

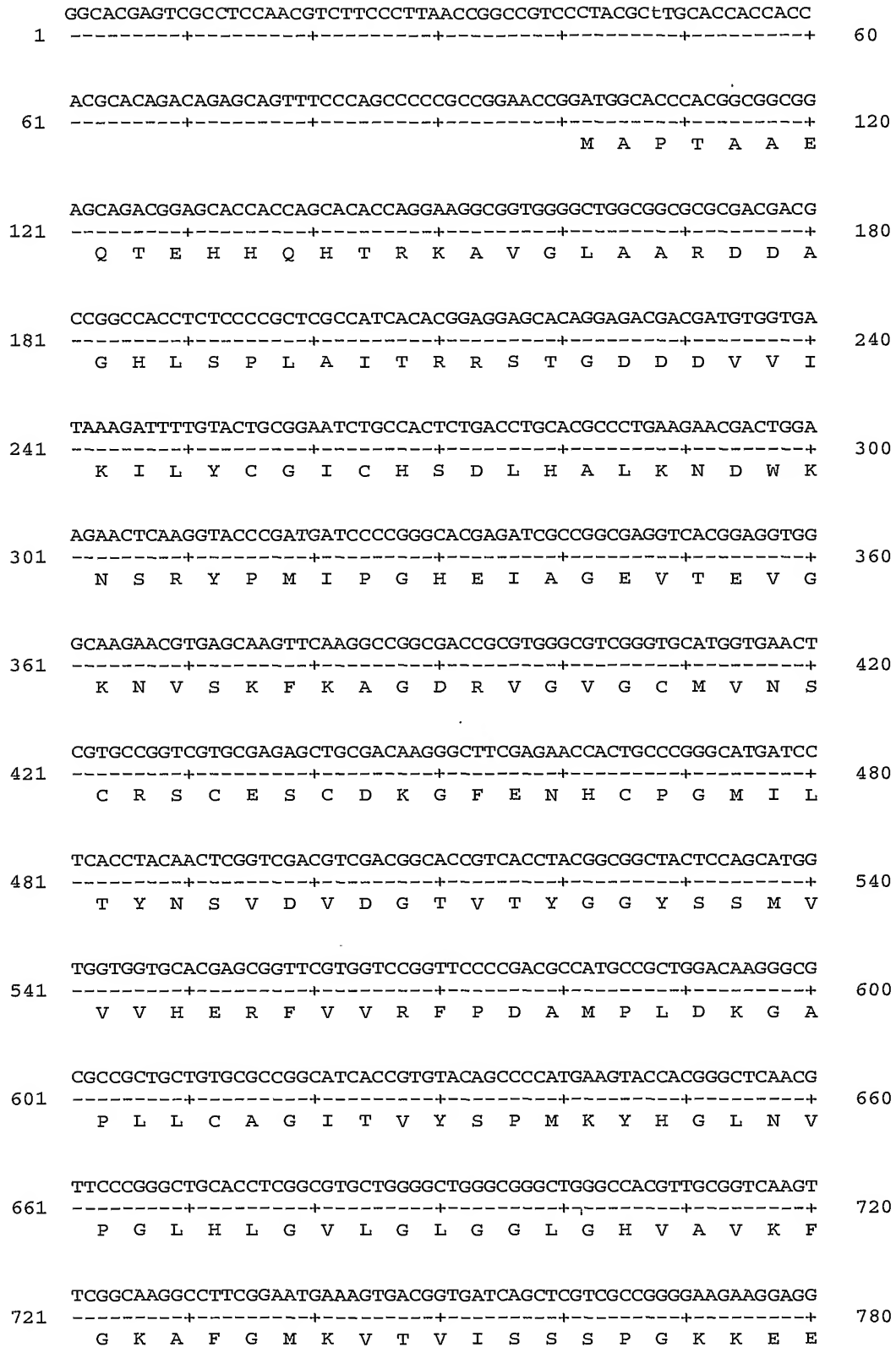

GGCACGAGTCGCCTCCAACGTCTTCCCTTAACCGGCCGTCCCTACGCTTGCACCACCACC
1 60
ACGCACAGACAGAGCAGTTTCCCAGCCCCCGCCGGAACCGGATGGCACCCACGGCGGCGG
61 120
M A P T A A E
AGCAGACGGAGCACCACCAGCACACCAGGAAGGCGGTGGGGCTGGCGGCGCGCGACGACG
121 180
Q T E H H Q H T R K A V G L A A R D D A
CCGGCCACCTCTCCCCGCTCGCCATCACACGGAGGAGCACAGGAGACGACGATGTGGTGA
181 240
G H L S P L A I T R R S T G D D D V V I
TAAAGATTTTGTACTGCGGAATCTGCCACTCTGACCTGCACGCCCTGAAGAACGACTGGA
241 300
K I L Y C G I C H S D L H A L K N D W K
AGAACTCAAGGTACCCGATGATCCCCGGGCACGAGATCGCCGGCGAGGTCACGGAGGTGG
301 360
N S R Y P M I P G H E I A G E V T E V G
GCAAGAACGTGAGCAAGTTCAAGGCCGGCGACCGCGTGGGCGTCGGGTGCATGGTGAACT
361 420
K N V S K F K A G D R V G V G C M V N S
CGTGCCGGTCGTGCGAGAGCTGCGACAAGGGCTTCGAGAACCACTGCCCGGGCATGATCC
421 480
C R S C E S C D K G F E N H C P G M I L
TCACCTACAACTCGGTCGACGTCGACGGCACCGTCACCTACGGCGGCTACTCCAGCATGG
481 540
T Y N S V D V D G T V T Y G G Y S S M V
TGGTGGTGCACGAGCGGTTCGTGGTCCGGTTCCCCGACGCCATGCCGCTGGACAAGGGCG
541 600
V V H E R F V V R F P D A M P L D K G A
CGCCGCTGCTGTGCGCCGGCATCACCGTGTACAGCCCCATGAAGTACCACGGGCTCAACG
601 660
P L L C A G I T V Y S P M K Y H G L N V
TTCCCGGGCTGCACCTCGGCGTGCTGGGGCTGGGCGGGCTGGGCCACGTTGCGGTCAAGT
661 720
P G L H L G V L G L G G L G H V A V K F
TCGGCAAGGCCTTCGGAATGAAAGTGACGGTGATCAGCTCGTCGCCGGGGAAGAAGGAGG
721 780
G K A F G M K V T V I S S S P G K K E E

FIGURE 27

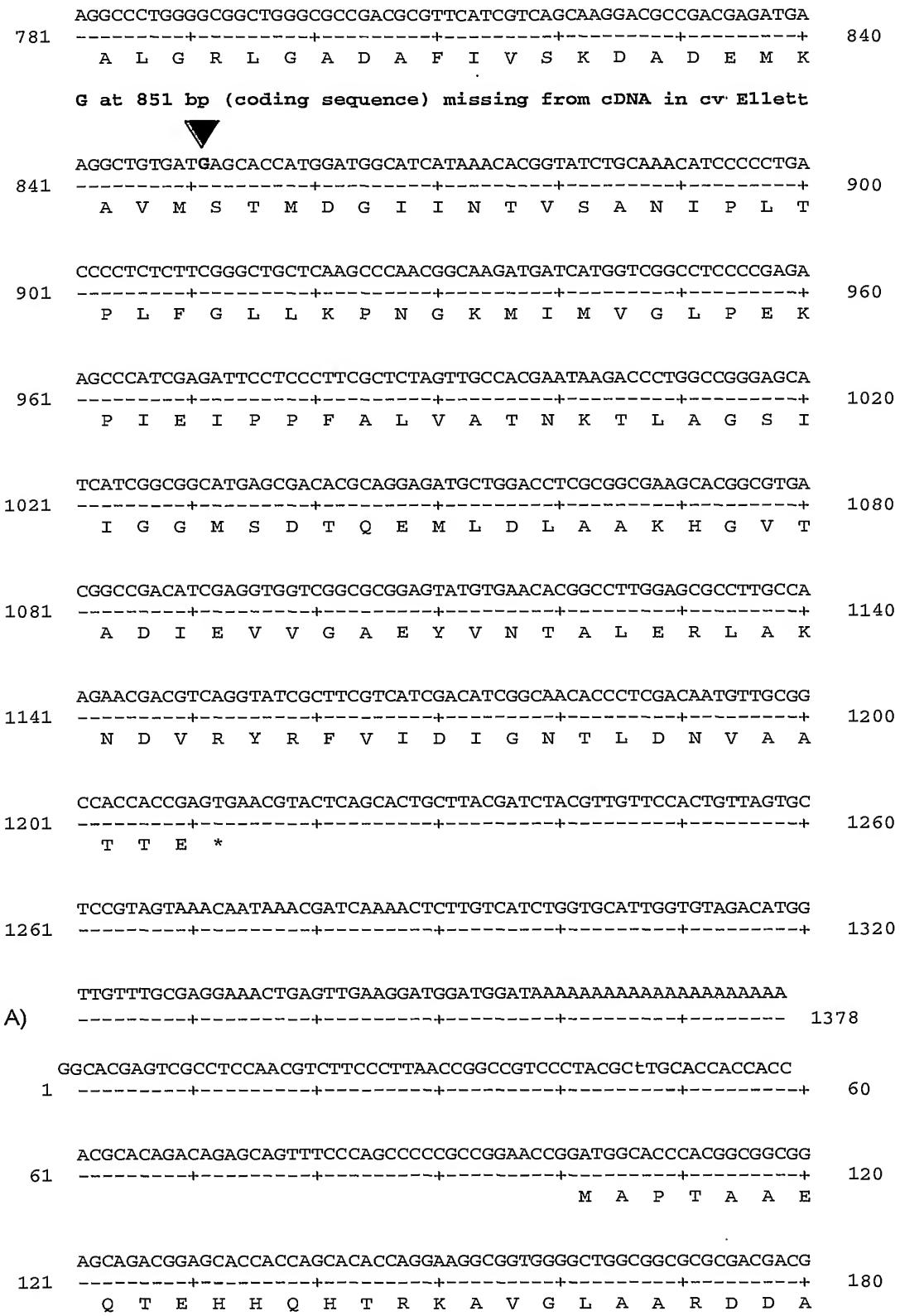


FIGURE 27 CONTINUED

```
    CCGGCCACCTCTCCCCGCTCGCCATCACACGGAGGAGCACAGGAGACGACGATGTGGTGA
181 ---------+---------+---------+---------+---------+---------+ 240
      G  H  L  S  P  L  A  I  T  R  R  S  T  G  D  D  D  V  V  I

    TAAAGATTTTGTACTGCGGAATCTGCCACTCTGACCTGCACGCCCTGAAGAACGACTGGA
241 ---------+---------+---------+---------+---------+---------+ 300
      K  I  L  Y  C  G  I  C  H  S  D  L  H  A  L  K  N  D  W  K

    AGAACTCAAGGTACCCGATGATCCCCGGGCACGAGATCGCCGGCGAGGTCACGGAGGTGG
301 ---------+---------+---------+---------+---------+---------+ 360
      N  S  R  Y  P  M  I  P  G  H  E  I  A  G  E  V  T  E  V  G

    GCAAGAACGTGAGCAAGTTCAAGGCCGGCGACCGCGTGGGCGTCGGGTGCATGGTGAACT
361 ---------+---------+---------+---------+---------+---------+ 420
      K  N  V  S  K  F  K  A  G  D  R  V  G  V  G  C  M  V  N  S

    CGTGCCGGTCGTGCGAGAGCTGCGACAAGGGCTTCGAGAACCACTGCCCGGGCATGATCC
421 ---------+---------+---------+---------+---------+---------+ 480
      C  R  S  C  E  S  C  D  K  G  F  E  N  H  C  P  G  M  I  L

    TCACCTACAACTCGGTCGACGTCGACGGCACCGTCACCTACGGCGGCTACTCCAGCATGG
481 ---------+---------+---------+---------+---------+---------+ 540
      T  Y  N  S  V  D  V  D  G  T  V  T  Y  G  G  Y  S  S  M  V

    TGGTGGTGCACGAGCGGTTCGTGGTCCGGTTCCCCGACGCCATGCCGCTGGACAAGGGCG
541 ---------+---------+---------+---------+---------+---------+ 600
      V  V  H  E  R  F  V  V  R  F  P  D  A  M  P  L  D  K  G  A

    CGCCGCTGCTGTGCGCCGGCATCACCGTGTACAGCCCCATGAAGTACCACGGGCTCAACG
601 ---------+---------+---------+---------+---------+---------+ 660
      P  L  L  C  A  G  I  T  V  Y  S  P  M  K  Y  H  G  L  N  V

    TTCCCGGGCTGCACCTCGGCGTGCTGGGGCTGGGCGGGCTGGGCCACGTTGCGGTCAAGT
661 ---------+---------+---------+---------+---------+---------+ 720
      P  G  L  H  L  G  V  L  G  L  G  G  L  G  H  V  A  V  K  F

    TCGGCAAGGCCTTCGGAATGAAAGTGACGGTGATCAGCTCGTCGCCGGGGAAGAAGGAGG
721 ---------+---------+---------+---------+---------+---------+ 780
      G  K  A  F  G  M  K  V  T  V  I  S  S  S  P  G  K  K  E  E

    AGGCCCTGGGGCGGCTGGGCGCCGACGCGTTCATCGTCAGCAAGGACGCCGACGAGATGA
781 ---------+---------+---------+---------+---------+---------+ 840
      A  L  G  R  L  G  A  D  A  F  I  V  S  K  D  A  D  E  M  K
```

G missing at 851 bp in the cDNA isolated from cv Ellett resulted in a premature stop codon (truncated CAD2)

```
    AGGCTGTGATAGCACCATGGATGGCATCATAAACACGGTATCTGCAAACATCCCCCTGAC
841 ---------+---------+---------+---------+---------+---------+ 900
      A  V  I  A  P  W  M  A  S  *
```

FIGURE 27 CONTINUED

```
                                                                    CCCTCTCTTCGGGCTGCTCAAGCCCAACGGCAAGATGATCATGGTCGGCCTCCCCGAGAA
 901  ---------+---------+---------+---------+---------+---------+   960

                                                                    GCCCATCGAGATTCCTCCCTTCGCTCTAGTTGCCACGAATAAGACCCTGGCCGGGAGCAT
 961  ---------+---------+---------+---------+---------+---------+  1020

                                                                    CATCGGCGGCATGAGCGACACGCAGGAGATGCTGGACCTCGCGGCGAAGCACGGCGTGAC
1021  ---------+---------+---------+---------+---------+---------+  1080

                                                                    GGCCGACATCGAGGTGGTCGGCGCGGAGTATGTGAACACGGCCTTGGAGCGCCTTGCCAA
1081  ---------+---------+---------+---------+---------+---------+  1140

                                                                    GAACGACGTCAGGTATCGCTTCGTCATCGACATCGGCAACACCCTCGACAATGTTGCGGC
1141  ---------+---------+---------+---------+---------+---------+  1200

                                                                    CACCACCGAGTGAACGTACTCAGCACTGCTTACGATCTACGTTGTTCCACTGTTAGTGCT
1201  ---------+---------+---------+---------+---------+---------+  1260

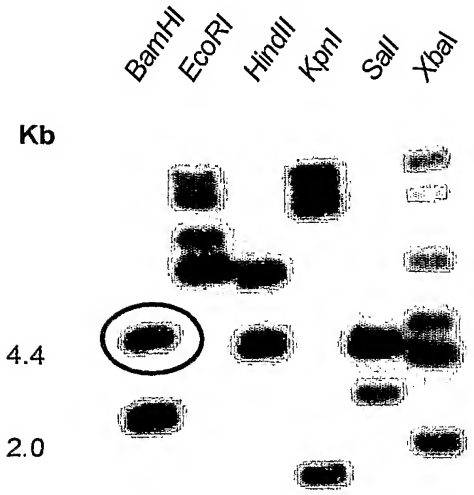
                                                                    CCGTAGTAAACAATAAACGATCAAAACTCTTGTCATCTGGTGCATTGGTGTAGACATGGT
1261  ---------+---------+---------+---------+---------+---------+  1320

                                                                    TGTTTGCGAGGAAACTGAGTTGAAGGATGGATGGATAAAAAAAAAAAAAAAAAAAAA
A)    ---------+---------+---------+---------+---------+-------  1377
```

FIGURE 27 CONTINUED

FIGURE 28

A


BamHI
EcoRI
HindII
KpnI
SalI
XbaI
Kb
4.4
2.0

B

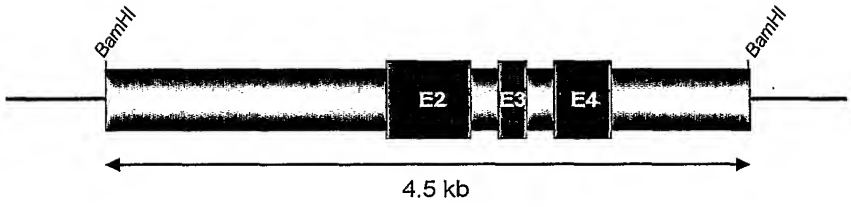
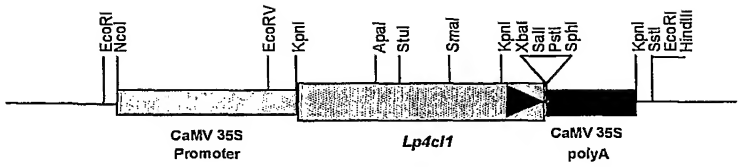

BamHI
BamHI
E2
E3
E4
4.5 kb

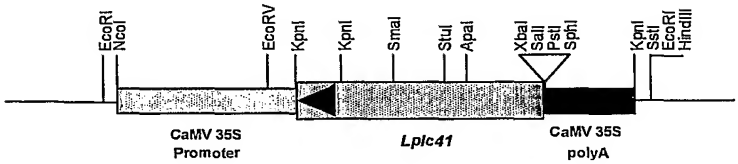
FIGURE 29A

A)

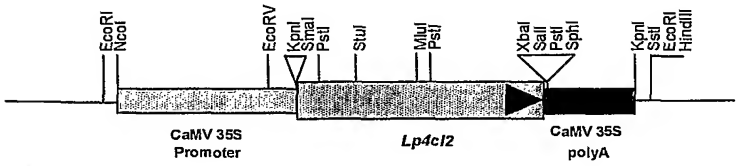
p35S4cl1



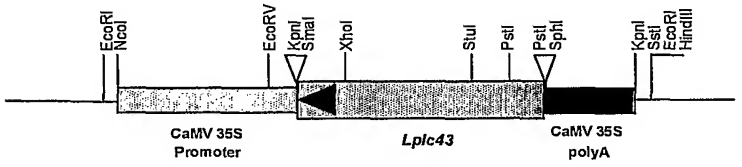
p35Slc41



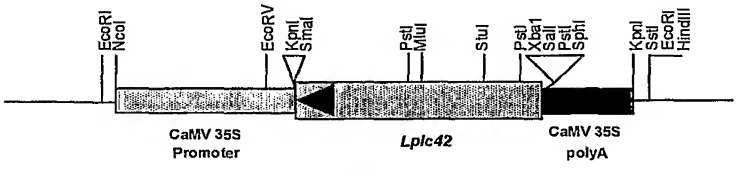
p35S4cl2



p35Slc43



p35Slc42



p35S4cl3

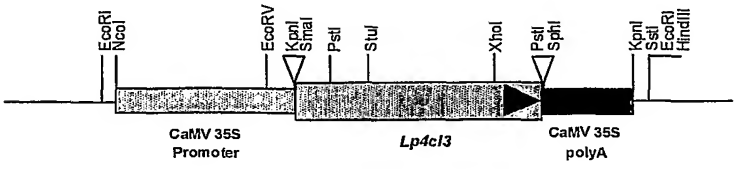
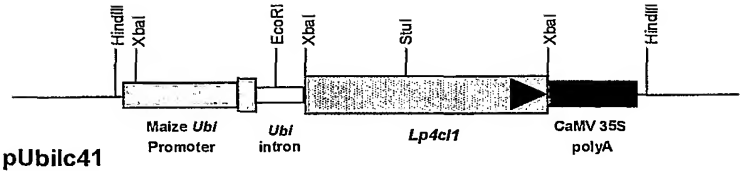


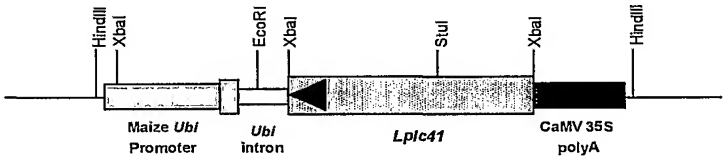
FIGURE 29B

B)

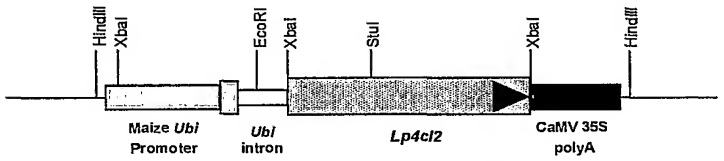
pUbi4cl1



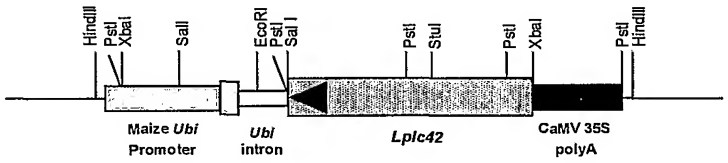
pUbilc41



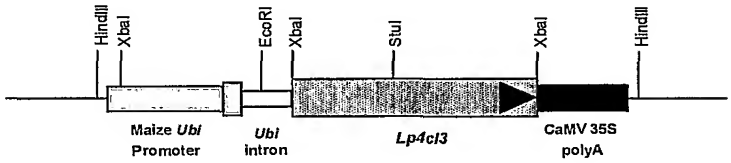
pUbi4cl2



pUbilc42



pUbi4cl3



pUbilc43

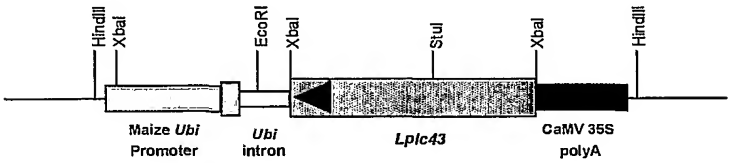
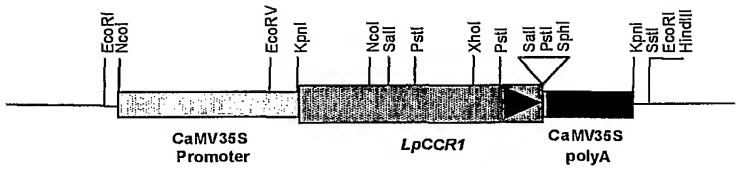


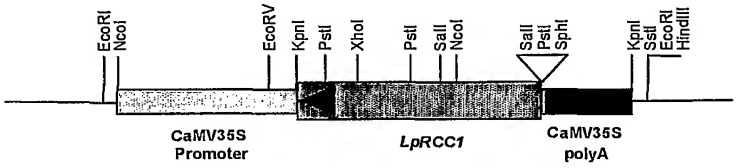
FIGURE 30

A)

p35SCCR1

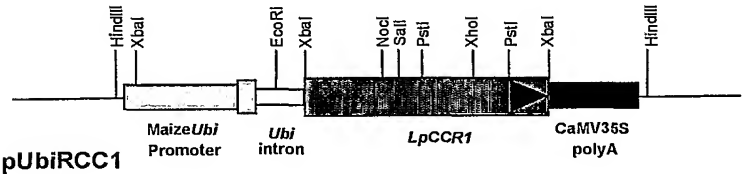


p35SRCC1



B)

pUbiCCR1



pUbiRCC1

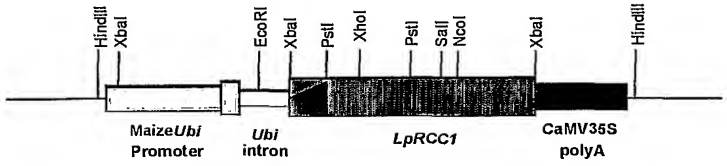
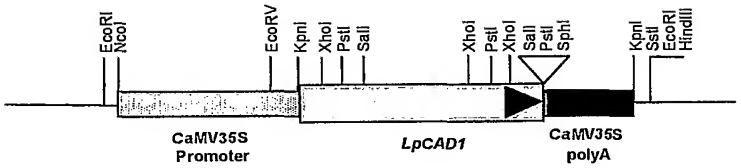


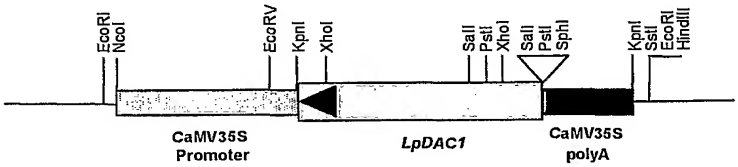
FIGURE 31

A)

p35SCAD1

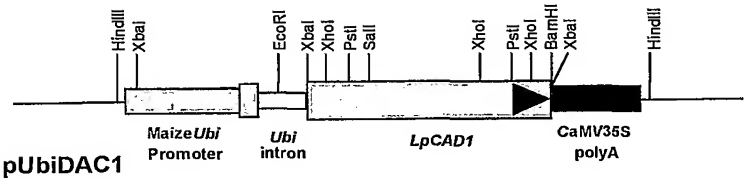


p35SDAC1



B)

pUbiCAD1



pUbiDAC1

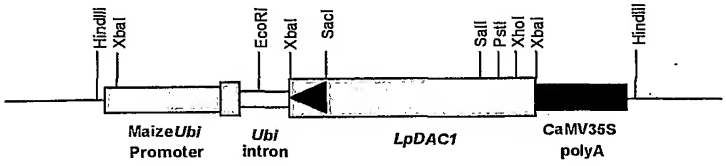


FIGURE 32

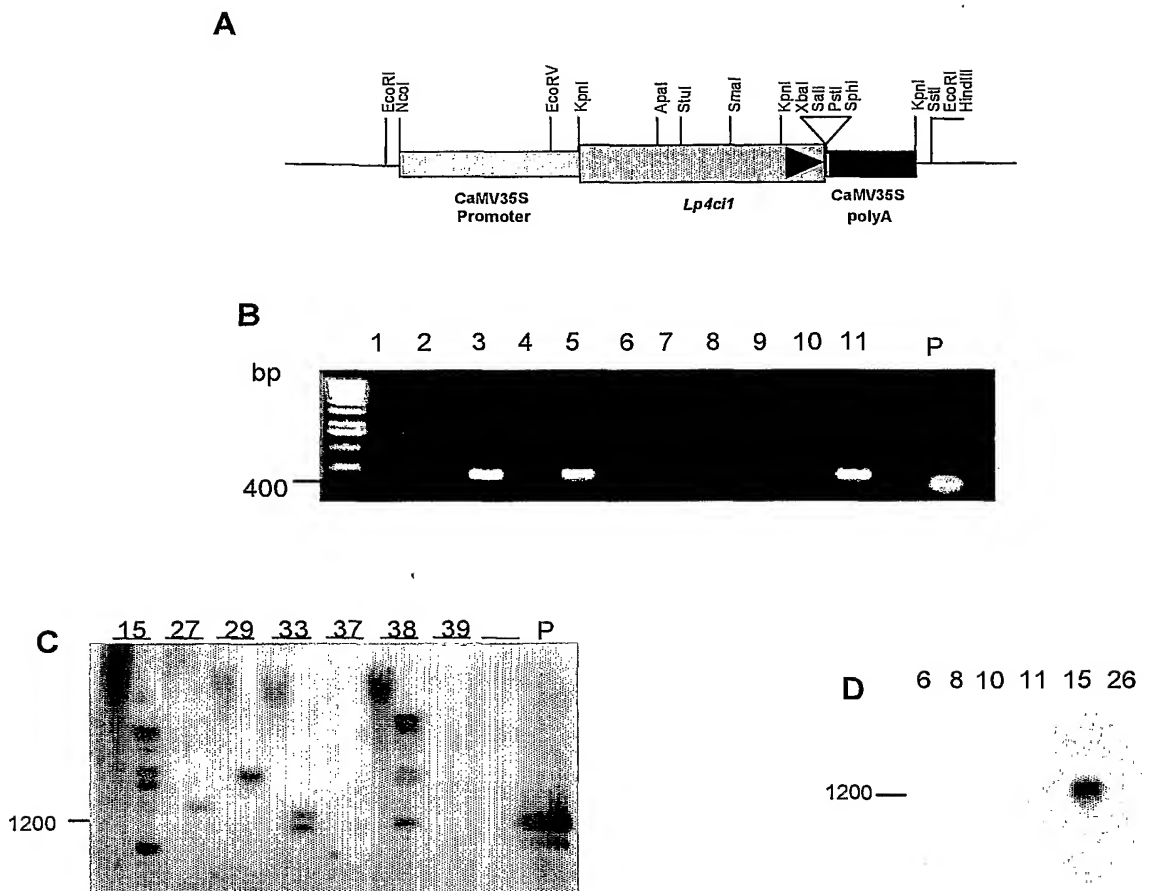

A
EcoRI
NcoI
EcoRV
KpnI
ApaI
StuI
SmaI
KpnI
XbaI
SalI
PstI
SphI
KpnI
SstI
EcoRI
HindIII
CaMV35S Promoter
Lp4ci1
CaMV35S polyA
B
1 2 3 4 5 6 7 8 9 10 11 P
bp
400
C
15 27 29 33 37 38 39 P
1200
D
6 8 10 11 15 26
1200

FIGURE 33

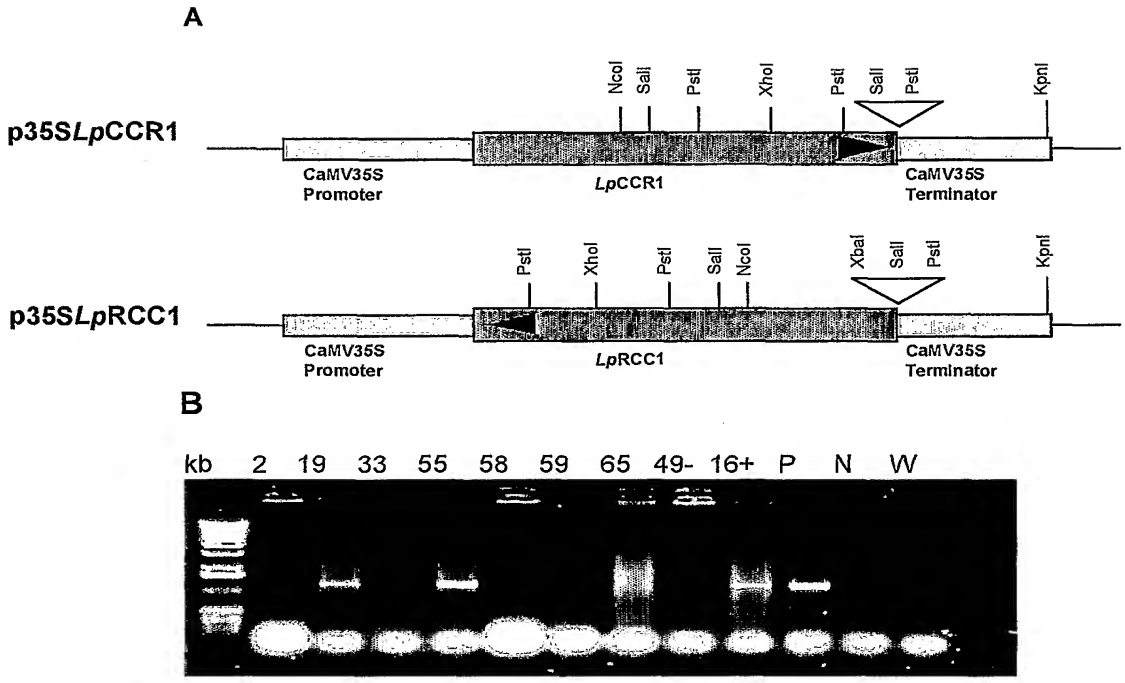

A
p35S*Lp*CCR1
NcoI
SalI
PstI
XhoI
PstI
SalI
PstI
KpnI
CaMV35S Promoter
*Lp*CCR1
CaMV35S Terminator
p35S*Lp*RCC1
PstI
XhoI
PstI
SalI
NcoI
XbaI
SalI
PstI
KpnI
CaMV35S Promoter
*Lp*RCC1
CaMV35S Terminator
B
kb 2 19 33 55 58 59 65 49- 16+ P N W

FIGURE 34

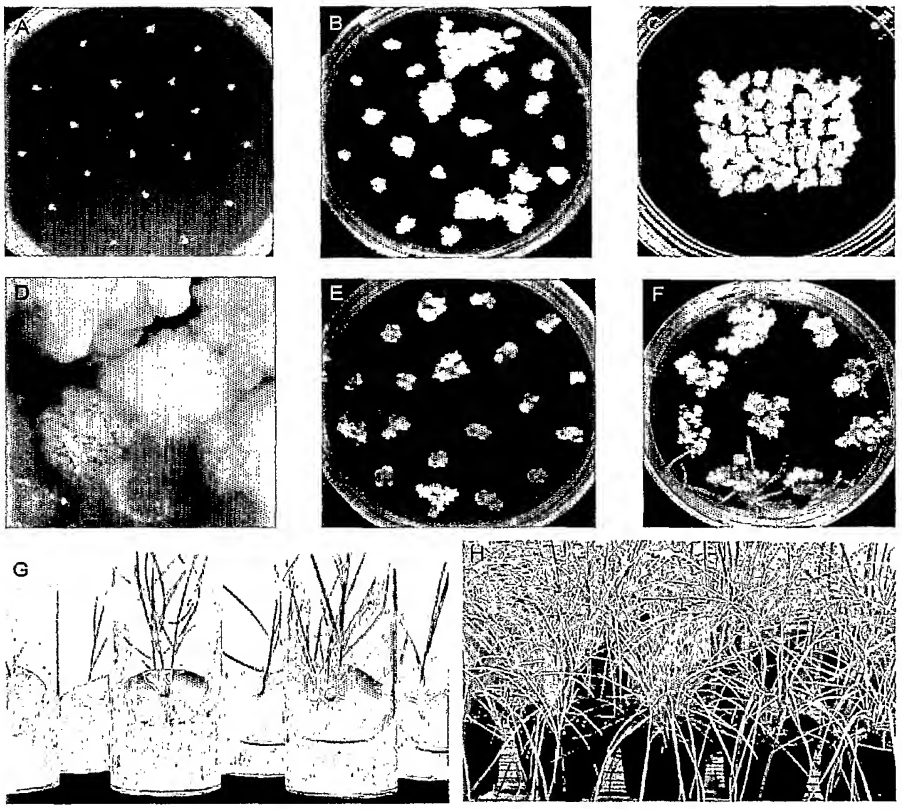
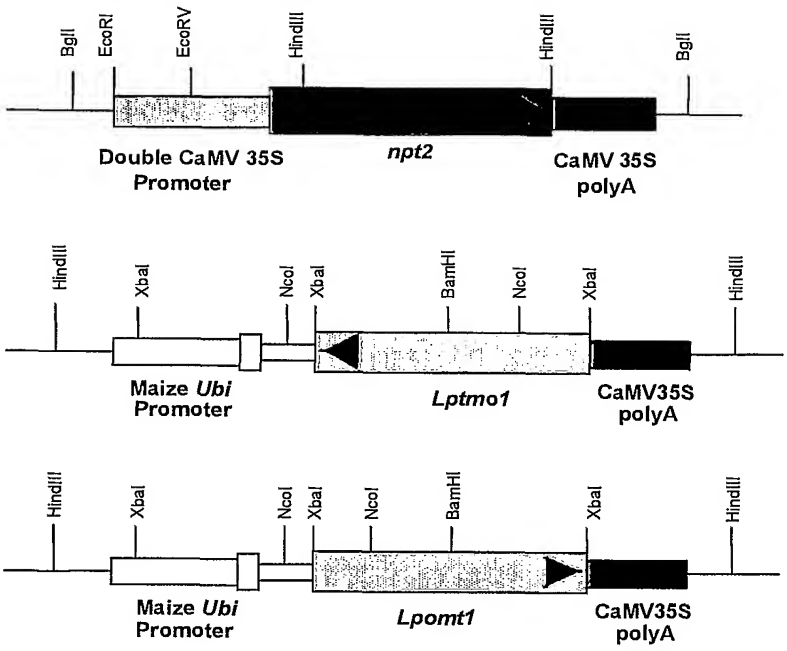
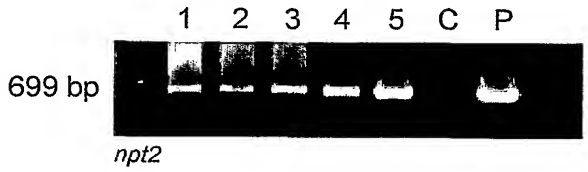


FIGURE 35

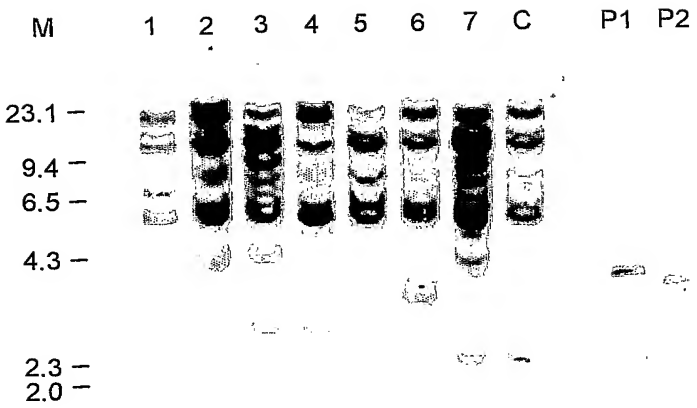
A


BglI
EcoRI
EcoRV
HindIII
HindIII
BglI
Double CaMV 35S Promoter
npt2
CaMV 35S polyA
HindIII
XbaI
NcoI
XbaI
BamHI
NcoI
XbaI
HindIII
Maize Ubi Promoter
Lptmo1
CaMV35S polyA
HindIII
XbaI
NcoI
XbaI
NcoI
BamHI
XbaI
HindIII
Maize Ubi Promoter
Lpomt1
CaMV35S polyA

B


1 2 3 4 5 C P
699 bp
npt2

C



D

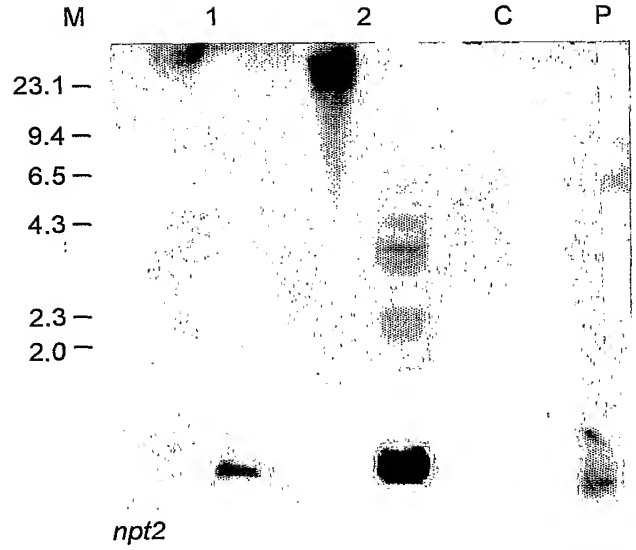


FIGURE 35
CONTINUED

E

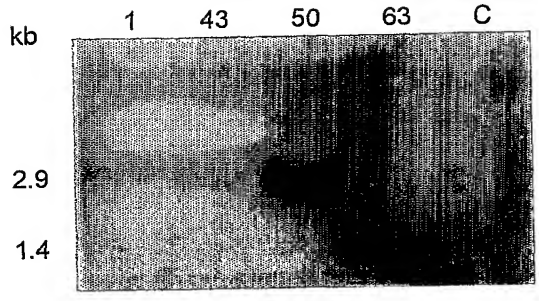
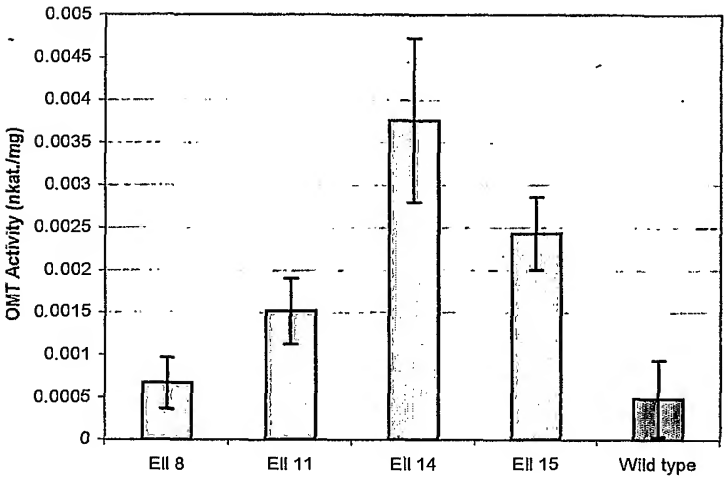


FIGURE 36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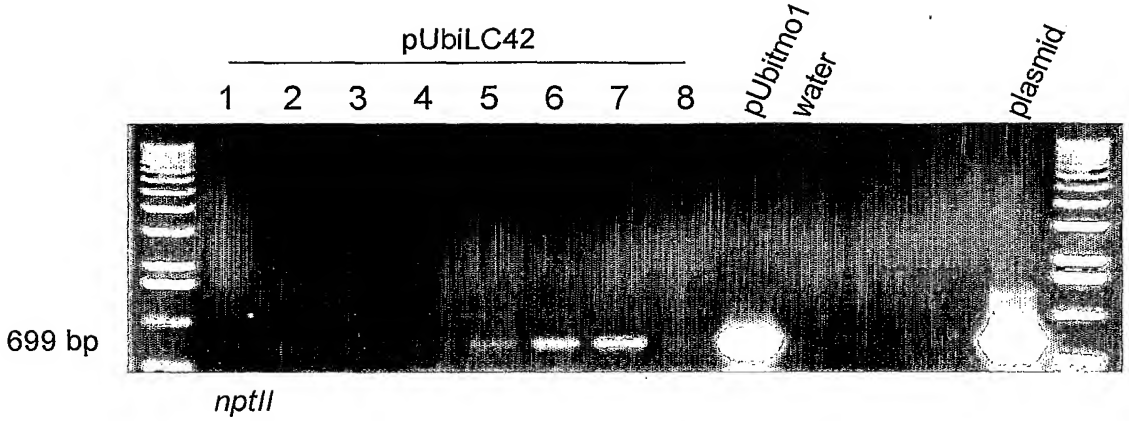
0.0045
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
OMT Activity (nkat./mg)
Ell 8
Ell 11
Ell 14
Ell 15
Wild type

FIGURE 37



```
                CGGGATCAACTTGGATGTCCTTTGCGGGCACGGTTTCAGGAACAACGACACATGCAGCAG
-2206  ------+---------+---------+---------+---------+---------+---------+---  -2147

                GGATCTCCTCCAAAGACTCACACAAAGGTGACATGAGCGCCCGCTTTTTTGAAGCCAAGT
-2146  ------+---------+---------+---------+---------+---------+---------+---  -2087

                TGGCTAAGAAATCGCAAAGCTTGGTGGAGTCGGCCACCTCAGGATCTGCAACAAAAGGCA
-2086  ------+---------+---------+---------+---------+---------+---------+---  -2027

                CCAAGGGAGCTGCCAACACATCAACCACAACATCATGTTCAAACGCAGTCTCCTCAAGCC
-2026  ------+---------+---------+---------+---------+---------+---------+---  -1967

                TCGAATGCTCAACCGAAAGAGAGGCAGAAGCTTCAACAAAAAACTCAGCCAACCCAAAGC
-1966  ------+---------+---------+---------+---------+---------+---------+---  -1907

                CCTCGACGTCATCAGAGATTAGGCTCTGAGGACCCGCAGGGAAGCAACCTTGTCAACAAC
-1906  ------+---------+---------+---------+---------+---------+---------+---  -1847

                CGCATCCGGCAGAAAAGGAGCAAGACCGGAGCAACCCTCAAGAGGCACACGAAAGACGTC
-1846  ------+---------+---------+---------+---------+---------+---------+---  -1787

                GAAGCCAAGAGGAGACGAGTCGCAGGGACGGCGGACAGGCGAGAAGGGGCCGTAGAACTC
-1786  ------+---------+---------+---------+---------+---------+---------+---  -1727

                CAAGAGCTCGGCGTCCCTCGACCTAGCATCCGAAGCACTGACCGGGGCACTCAATGCATA
-1726  ------+---------+---------+---------+---------+---------+---------+---  -1667

                ACTTTATCTTGATGGCATATGTACTCAAACCCATACAATGTTCACCATGCATTATCTATG
-1666  ------+---------+---------+---------+---------+---------+---------+---  -1607

                GAACATTCCTTCATATACAACTtCTGAGTGGTCAGTGCATAGGAATTTTCATTAACAACC
-1606  ------+---------+---------+---------+---------+---------+---------+---  -1547

                AAAAACATACTTGGGGCCTACACACACTTTCACAGCATGGAAAACTTGTTAGCTTTTTAA
-1546  ------+---------+---------+---------+---------+---------+---------+---  -1487

                AGAGTTGCAAAATCTGTCAAGCGAATGTTCTTGTGATAATTGGAACGAAGCATGTTTCCC
-1486  ------+---------+---------+---------+---------+---------+---------+---  -1427

                CATTTTCAATGTGTGTCTCTTACCCTAACTAGCACCCGACCAACAAAATCTGACCATCCT
-1426  ------+---------+---------+---------+---------+---------+---------+---  -1367
```

FIGURE 38

```
                AGTTATATCATCATAGAGACCCACATGTAGGTTGACCCCCATAACACTTGTGTGGATATC
-1366  ------+---------+---------+---------+---------+---------+---  -1307

                ATGGAAAATGGCCTTGATCAACACTTTCTTTCCTACTTGGTACAAATGGTTATGGACTTA
-1306  ------+---------+---------+---------+---------+---------+---  -1247

                CTCAATTAGTGCTTTAGAGAGCTTTGGCTGCAGACTTTGTAGCTTCCCAATATTCATAGG
-1246  ------+---------+---------+---------+---------+---------+---  -1187

                TCCCTCCGGAGTGGGCAGCCCCATCTACATAGGCTCAAAACCAGATTTTTGTAACATGTT
-1186  ------+---------+---------+---------+---------+---------+---  -1127

                AGACACTTTCAACTTCATCATAGACCATCAAGGAGCTGGCATGTGACAGTGATATATGTA
-1126  ------+---------+---------+---------+---------+---------+---  -1067

                TCAATTACCCATTCAACACGAATAGCTTGCTCATGCATGGTTAGTCTTGCGGCGGCGGGG
-1066  ------+---------+---------+---------+---------+---------+---  -1007

                CGGGACCATCGAACACACCGCCGGGCGGTCAGTAGGCTAGGGTTAGATAAAATCTAGCCG
-1006  ------+---------+---------+---------+---------+---------+---  -947

                TTTTCATTCAAACTTGTGATATATAATCAAATTTAAATAAAAACCTTTATTTTCGTGCAT
 -946  ------+---------+---------+---------+---------+---------+---  -887

                TTTTATTTATTTGAGGGCGTGTTTGGGGGACACGGCTGGAAAGTGACATCCCCAAACACT
 -886  ------+---------+---------+---------+---------+---------+---  -827

                GCACGAAGAAAACGCGTCGCCAAAAAATTCGATCCGGCGTCAGTCCTTTGGGAGACGATT
 -826  ------+---------+---------+---------+---------+---------+---  -767

                TGGATGACGCGGCTAGAGATGCTCTAAGTTCTCCACGCCATGTTTCTTTCTATATATACA
 -766  ------+---------+---------+---------+---------+---------+---  -707

                CACAGCCCAAGGTCCATGAAAAGTAAAACGGCACGACGACACGCACCGGCGACAACTTCA
 -706  ------+---------+---------+---------+---------+---------+---  -647

                CATTACGGCACATCGCTATTACGGACCACATACAACTCCACCGCTATTCTCAGCCAAGTC
 -646  ------+---------+---------+---------+---------+---------+---  -587

                ATACATGACATGATCCAATGGACGACTTTGTGAGCGAAACTAGAACCTTGCGGGGTTTAG
 -586  ------+---------+---------+---------+---------+---------+---  -527

                ATTTTCCAATGTGGATAAGTTGTACGCGCCGACTAGCTTTACACTTGGTTGAAAAAAGCT
 -526  ------+---------+---------+---------+---------+---------+---  -467
```

FIGURE 38 CONTINUED

atactcctactgtatattggtaaaacaaaacattttcttttttatttgataggagtgctgcaaattaaagttctttgtgtca
tttttcaaaggaaaaaaaaaacacctttaccactcttcttccttgccatcatttttttttttaccaaagtttgttctgtcaaa
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SequenceDescription :

Custom Codon

Sequence Name : CCR1genomicseq (SEQ ID NO: 18)

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      TATTGTAGCACGACTTCTCACTGACATAGGAATGTAAACAGTCTCTCCACGCCATGTTTC
-466  ------+---------+---------+---------+---------+---------+---  -407

      TTTCTAGTAGTAGCATACTAGTAGTAACTTCTCTTTGTCCTACACACACCCAGGGTCCAA
-406  ------+---------+---------+---------+---------+---------+---  -347

      GAAAGGAAAACGGCACGACGGCACCCACCGACGACGACGACTCCACATCACGGTTCGGTA
-346  ------+---------+---------+---------+---------+---------+---  -287

      AAAAAAGTCAAAACTCGCTGACGTGGCACCACCGGTCGCAGTCAACTGACGCGCTCCTCT
-286  ------+---------+---------+---------+---------+---------+---  -227

      GCGCAGGTyTCACTtCAAGTTTCACCTACCACTGTGGGCCCACCGCCAaTGTGGGCCCCG
-226  ------+---------+---------+---------+---------+---------+---  -167

      CGAGCTtCTtACTCACTGACCTGTCTCCCACCAGCCTCCTCGCCGGTATATTACCCCGGC
-166  ------+---------+---------+---------+---------+---------+---  -107

      CCCCAATTTCCTCTGCCTTCCCACGAGCAGCAGCCGGAGCACGGAATCCCGGCCGCCATT
-106  ------+---------+---------+---------+---------+---------+---  -47

      CCTCCACCTTCAGCTCCGCCCAAAGATTTCCATCCGGCGAGATCCATGGGCTCCATCGCG
 -46  ------+---------+---------+---------+---------+---------+---  13
                                                  M  G  S  I  A

      GCGGACGCGCCTCCCGCGGAGCTGGTGTTCCGGTCCAAGCTCCCGGACATCGAGATCCCG
  14  ------+---------+---------+---------+---------+---------+---  73
      A  D  A  P  P  A  E  L  V  F  R  S  K  L  P  D  I  E  I  P

      ACCCACCTGACGCTGCAGGACTACTGCTTCCAGCGCCTGCCGGAGCTCTCCGCGCGCGCC
  74  ------+---------+---------+---------+---------+---------+---  133
      T  H  L  T  L  Q  D  Y  C  F  Q  R  L  P  E  L  S  A  R  A

      TGCCTCATCGACGGCGCCACGGGCGCCGCGCTCACCTACGCCGACGTGGACGCCCTCACG
 134  ------+---------+---------+---------+---------+---------+---  193
      C  L  I  D  G  A  T  G  A  A  L  T  Y  A  D  V  D  A  L  T

      CGCCGCTGCGCCGCGGGCCTCCGCCGCCTGGGGGTCCGCAAGGGCGACGTCGTCATGGCG
 194  ------+---------+---------+---------+---------+---------+---  253
      R  R  C  A  A  G  L  R  R  L  G  V  R  K  G  D  V  V  M  A

      CTGCTCCGCAACTGCCCCGAGTTCGCCTTCGTGTTCCTCGGCGCCGCCCGGCTCGGCGCC
 254  ------+---------+---------+---------+---------+---------+---  313
      L  L  R  N  C  P  E  F  A  F  V  F  L  G  A  A  R  L  G  A
```

FIGURE 38 CONTINUED

```
     GCCACCACCACCGCCAACCCGTTCTACACGCCCCACGAGATCCACCGCCAGGCGACCGCC
314  ------+---------+---------+---------+---------+---------+---  373
     A  T  T  T  A  N  P  F  Y  T  P  H  E  I  H  R  Q  A  T  A

     GCCGGGGCCAGGGTCATCGTCACCGAGGCCTGCGCCGTCGAGAAGGTGCGCGCCTTCGCC
374  ------+---------+---------+---------+---------+---------+---  433
     A  G  A  R  V  I  V  T  E  A  C  A  V  E  K  V  R  A  F  A

     GCCGAGAGAG
434  ------+---  443
     A  E  R
```

FIGURE 38 CONTINUED

```
                TCGACGCGGCCGCGTAATACGACTCACTATAGGGCGAAGAATTCGGATCATATGGATTCG
-6735  -----+----------+---------+---------+---------+---------+----  -6676

                ACACTGGAATTTACTCCCATCGGGAGCGTGCAAACAAAAAGGTGTTATAGCAAGAAGACA
-6675  -----+----------+---------+---------+---------+---------+----  -6616

                CTGGCAACATTGCCAGCACAGAATTTGTTACAATCATAGAAAGTTTTATGACAGGACATT
-6615  -----+----------+---------+---------+---------+---------+----  -6556

                GTTTCAACCGAAAGCAAGATTACAACAATATAATCAAGGGCTTGGGTCTGGTTGGACATG
-6555  -----+----------+---------+---------+---------+---------+----  -6496

                CTCGGTCCAATGGACGATTTATTTGCCGAGACCAGCTCAAGGAGTTGACGAGCACACTTA
-6495  -----+----------+---------+---------+---------+---------+----  -6436

                AGCGCCGAGATCTTAAAGGCACCCAAGTCAACAAGTCGCCCATCTTGCTCTTTTGGCAGC
-6435  -----+----------+---------+---------+---------+---------+----  -6376

                TCCTTGGACATCTCTTCGATATTGGCTTTGAAGCCATGACCCATCATAAGCTGAAAGGCT
-6375  -----+----------+---------+---------+---------+---------+----  -6316

                AGGAGGGCACCATAGGTACGCGAAGTACGTTTGAATACCTCGAGGACCTCCCTCGTGTTG
-6315  -----+----------+---------+---------+---------+---------+----  -6256

                ATGGCGAAAGCATCGATCAGCTGCCCCAAGGTCTTGTTTTGATCGATCTTGGGGAAGATC
-6255  -----+----------+---------+---------+---------+---------+----  -6196

                ATCGAGTGCATCCGCGTCATGGATCCTTTACCCTTCTGAAGGAGGTCCTGAAAAAGCTGG
-6195  -----+----------+---------+---------+---------+---------+----  -6136

                TGAGACCCGAGGGTCATTGACAAAGCATTCGCCGGAGAATTATTCGGCAATTTATCTAGA
-6135  -----+----------+---------+---------+---------+---------+----  -6076

                GCCTCAGCAGGGATGTAGGCAGCTTCTGGAGAAAGTGAAAGAGGAGGAGCTCACTAACCA
-6075  -----+----------+---------+---------+---------+---------+----  -6016

                AAATCAAATCGATAAAGCAAAAATCGGAAAGGAGGCCAAAAGGGGATTACTGAGCAAGGC
-6015  -----+----------+---------+---------+---------+---------+----  -5956

                CAAGGAAGATTGGCGAAGGAGCTCATCTTTTTCAATCGCCCGAGCTTCGGCAGCAAGCCT
-5955  -----+----------+---------+---------+---------+---------+----  -5896

                GGATGCCTCTTCATCCTTCAGCCTCTTTCTTAGCCCCTCGAGCTCATCCTTAAAGGAATC
-5895  -----+----------+---------+---------+---------+---------+----  -5836

                AACCTCCTGGCGGGCCTCGGCAGCTATCTTTATCGCACCCTCCAGCTTCGAGGAAGAAGA
-5835  -----+----------+---------+---------+---------+---------+----  -5776

                CTCGACCTCCTTTTGCAGCCGAGTCTTGTCAACTTCCAGAGAAGTGTATTGGGAGGCGAA
-5775  -----+----------+---------+---------+---------+---------+----  -5716

                GGCCTCCAGAGAAGAGATAACAGCTCACAAATCCTTAAGAGATAAGGAAAAATAATTAGA
-5715  -----+----------+---------+---------+---------+---------+----  -5656

                CGAAGAACTGGTTGTCAACAAACTTATAATTTGATCAGGGAAATCGTCCCACATGGATAT
-5655  -----+----------+---------+---------+---------+---------+----  -5596

                ATCGTTAAAACAGGAAAAGCTTACAGGTTTCCCTGGAGGAGAAGCTGTAACCACGGCAGT
-5595  -----+----------+---------+---------+---------+---------+----  -5536
```

FIGURE 39

```
                CAAAGAAATCTCCTTCCCTTTGGAAAGGGAAGAAGTTGTCGATATTTGAGCCATGGGGGC
-5535  -----+---------+---------+---------+---------+---------+---------+----  -5476

                TGCGGCAGGAGTCGAAGCCTCGGAAGCGGCTGGATTCGGCACGATGGCACCAGATTTGGC
-5475  -----+---------+---------+---------+---------+---------+---------+----  -5416

                CTTCTTGGCCGGAGGCTCGATGAAGCCATCTTCACTGCAAGAACAAAAAACTAGCGAAGT
-5415  -----+---------+---------+---------+---------+---------+---------+----  -5356

                CAGAATTCAATGCATATGGCGAAGTTAGAACACAATCCTGGAAAAGGAAGCAAGGACTTA
-5355  -----+---------+---------+---------+---------+---------+---------+----  -5296

                CAATTCATAGAGACCATCTTCATCGGCAAAGCCGCCGGATGATCTCTTTGGAGGTAGTGC
-5295  -----+---------+---------+---------+---------+---------+---------+----  -5236

                CTCGGCCTTTTCCGTAGCTGCATCAACAAAGGCAGCACGATCAGCATCGTCATCATGCAT
-5235  -----+---------+---------+---------+---------+---------+---------+----  -5176

                TGACCCCGCTGTATCGCTCATATCATCGGCAGAGAATCGAGGATTGATGGAAAAAGCCTC
-5175  -----+---------+---------+---------+---------+---------+---------+----  -5116

                AGGATTCATCGGATCATCATGTTGATCTATCGGGCTTGCATTCCCTAGAGTATGGGACCC
-5115  -----+---------+---------+---------+---------+---------+---------+----  -5056

                TACAAGGACTAAGGAATCCCTTTTCTTGGAAAAATTGTTCGACAGGTCTTGCAAACGTTC
-5055  -----+---------+---------+---------+---------+---------+---------+----  -4996

                AAGAGCCGTAAGGATCTGTCGTAGTTGACGAGTGAGAATAATGGCAGTTAAAATAATCAA
-4995  -----+---------+---------+---------+---------+---------+---------+----  -4936

                AGGAACATGACAATAAGAGCATAAAGGGGAAATTTACCTCGGTTGGCAGATGACCAGCGT
-4935  -----+---------+---------+---------+---------+---------+---------+----  -4876

                CAAATGGCGGTTGAGGAGATATCAGTGGAATTGAATCTTCCTGGCTAAAGAGGGTGAGAC
-4875  -----+---------+---------+---------+---------+---------+---------+----  -4816

                ACCGGACTTCGTCAAGCAGTTCTTTTTCGGATAATTCAGCAATATTTACTCTAGTCTCGT
-4815  -----+---------+---------+---------+---------+---------+---------+----  -4756

                CCCTGGGACCCGAATACAACCACATCGGATGGGTCCTAGACATGATCGGCTGAACTCGAT
-4755  -----+---------+---------+---------+---------+---------+---------+----  -4696

                GTTTTAAGAACACAGCGGCTACCTCAGTACCTATCATGGTTTGACCATCGGATTCTTTGA
-4695  -----+---------+---------+---------+---------+---------+---------+----  -4636

                TCCGAAGGAATCTATCAAATAACTTGTCTACTGTTGGTTTTTCATCGGGTGAGAGGATAT
-4635  -----+---------+---------+---------+---------+---------+---------+----  -4576

                TTTTCCAAGACTTCTTGGGCTTGCTTCTAGAACATCGGAGAATTGGGGGGGAGCTGGGAG
-4575  -----+---------+---------+---------+---------+---------+---------+----  -4516

                TCGGCTGCTGATGAGTCCTTAATATAAAACCACTTCAGCCTCCAGCCTTGCACGGATTCT
-4515  -----+---------+---------+---------+---------+---------+---------+----  -4456

                TTCATCGGGAAGTTGAAGTAGTTGACTTCCTTACGAGCAACAAAACCAACCCCACCAATG
-4455  -----+---------+---------+---------+---------+---------+---------+----  -4396
```

FIGURE 39 CONTINUED

```
                ACGAAGGACCCACCACTGCTGTTATATCTTTTCACGAAGAAAATCTTCTTCCACAAACCA
-4395           -----+---------+---------+---------+---------+---------+----   -4336

                AAGTGGGGCTCAATGCCCAAAAACGCTTCGCAGAGGGTGATAAAGATGGCAAGGTGAAGG
-4335           -----+---------+---------+---------+---------+---------+----   -4276

                ATTGAGTTGGGGGTTAACTTCCATAATTGAATCTCATACACTCGAAGGAGGTGGTGAAGA
-4275           -----+---------+---------+---------+---------+---------+----   -4216

                AATTTGTGAGCGGGAAGCGAAAGACCTCGGTACAAGAAGGATAAGAACATCACAGTAAAA
-4215           -----+---------+---------+---------+---------+---------+----   -4156

                CCGGCAGGAGGATTGGGCCGTGAAATTGCACCTGGAAGAATAACATTCCCCTCGTCAGAA
-4155           -----+---------+---------+---------+---------+---------+----   -4096

                GAAATTATTACGAGGCTCCGGGCCCTCTTTTCATCTCGCTTCGTGGTGGTAGAAGCTGGC
-4095           -----+---------+---------+---------+---------+---------+----   -4036

                CAATCGCCAGGGATAGGCCCGGCCGTGGAGCTTGACGGCGCTGGCGGTGCCGGAGCTGAG
-4035           -----+---------+---------+---------+---------+---------+----   -3976

                GGAGGAGCATCTGGCGCGCTTCTCCGCGGCGGATTCGAAGGAGCCCTGACGGTGGTGCCA
-3975           -----+---------+---------+---------+---------+---------+----   -3916

                CTGCTCACGGCGCTGGTGGCGAGAGTGGGATTCTTCTTCTTCACCATTGTGAGATTTGAG
-3915           -----+---------+---------+---------+---------+---------+----   -3856

                GGAGATCTGGGAGTTGCGACGGTGGCGTGGTAGTTGCAAACGAAAAGGATGAATGAGGAA
-3855           -----+---------+---------+---------+---------+---------+----   -3796

                GAAGGGACGCAAGGATGAAGTGTGGAAAGGGGAGTTTACCCCAAGAGATTATAAAGTGAA
-3795           -----+---------+---------+---------+---------+---------+----   -3736

                AGGAAAACCTGAGAATTGAGCGGGCACGTGTCGTTGCTCTCAATTTATTGAGGGGATTTT
-3735           -----+---------+---------+---------+---------+---------+----   -3676

                TTCTCATCATAGATCGCGGAAATCGAGGAGTCACCTTGGTAACTGCACGCAAGTAGTGGT
-3675           -----+---------+---------+---------+---------+---------+----   -3616

                CATTTCTTAAACAGAACCGCATAGAAGTAGGATGGGACCGTCAGGTCACGTCCTATCAGT
-3615           -----+---------+---------+---------+---------+---------+----   -3556

                CAGATTTACAACAGTAATTACATCATCACTGACGTCAAAGTATGCTTGAAGTATCCGAAG
-3555           -----+---------+---------+---------+---------+---------+----   -3496

                AAAAGTCGAAATTTGGGCTCGAAGACTTTCTTGCAGAGAAGCGCGTGAAAGGAATATCTA
-3495           -----+---------+---------+---------+---------+---------+----   -3436

                AGGAAAGGGTCAAAACATTCGGCTCGAGTCTACGCACGGATTGCAAGCATCCGTACCTAG
-3435           -----+---------+---------+---------+---------+---------+----   -3376

                ACTCGGGGGCTACTCCCATCGGGAGCGCTGGACGTGCACCCGATAAATTTAGACGAGGAT
-3375           -----+---------+---------+---------+---------+---------+----   -3316

                GAAAACCGGAAACCCAAGTGCTACTCCCATCGGGAGCGCCGATTACGCACCCGACAAACT
-3315           -----+---------+---------+---------+---------+---------+----   -3256

                TTTTTGCACTCCAGGATCATGCCCGGGGACTTAATTCTGTGTAGAGTAGCGTTGTTTTGT
-3255           -----+---------+---------+---------+---------+---------+----   -3196
```

FIGURE 39 CONTINUED

```
                CTTCGGCAGTTAACCAGCAAAGCTGGACACGTTACTCAATATCCTTTACGCATTAAACCC
-3195           -----+---------+---------+---------+---------+---------+----  -3136

                TTACTTGAAGAATTGAAGCCCCGATGCAAATATATCGGATGACCTATGAAGGCCTGCGGA
-3135           -----+---------+---------+---------+---------+---------+----  -3076

                AAGCTTCGGGAGAAGAAGACATTCGAGTGGCACAACTTGAGTCTACGAACGGATTGCAAG
-3075           -----+---------+---------+---------+---------+---------+----  -3016

                CATCCGTACCTAGACTCGGGGGCTACTCCCATCGGGAGCGCTGGACTCGCACCCGATAGA
-3015           -----+---------+---------+---------+---------+---------+----  -2956

                AGGAGATGATGATATTACAAGAAGGACAAGAAGTATCAAGGGAGAAGAACATTCGGTGGA
-2955           -----+---------+---------+---------+---------+---------+----  -2896

                GGCATGCTTTAGTCTCTACCCGAAAAAACTTCGGCTAGACACTCGGGGGGCTACTGACGT
-2895           -----+---------+---------+---------+---------+---------+----  -2836

                GGGCATTACCCTTCGGGTAACTGATATTGCCCTATCCTGTACGACCCAACTGGAGGCCCA
-2835           -----+---------+---------+---------+---------+---------+----  -2776

                TGAAGACACTCGAAGGCAAGGTGGACCACTACGTCGGTGCCGAAGGGGGTTCCTTGAAGA
-2775           -----+---------+---------+---------+---------+---------+----  -2716

                ACAAGACGAAGAAAAGAAGAATACAAGAAAAGTATAGAACTAGGATCTTTTGTAACCTGG
-2715           -----+---------+---------+---------+---------+---------+----  -2656

                TCGTACCCGGACAGATCTCTCGAGACCTGGCCCCCTACATATGGGCTAGGAGAGGGGCTG
-2655           -----+---------+---------+---------+---------+---------+----  -2596

                CCGAGAGGGACACACACAATCTTAGCAATTTTAGCCACCATAAGTCCAGAGCAAGGTCCC
-2595           -----+---------+---------+---------+---------+---------+----  -2536

                CGTAGAACTTAGCCTCTCGACGAGATCACAGCCGAAACCTTCGGCACCCCATTGTAACCC
-2535           -----+---------+---------+---------+---------+---------+----  -2476

                GATATTTTCATAGTCAAGATCAGACAGGTAGGACGTAAGGGTTTTACCTCATCGAGGGCC
-2475           -----+---------+---------+---------+---------+---------+----  -2416

                CCGAACCTGGGTAAATCGCTCTCCCCGCTTGTTTGATAACCGATGGCTTGTGTCAGCTTA
-2415           -----+---------+---------+---------+---------+---------+----  -2356

                CATGATTCCATCTACCCTAAACCTCAAACGGAGGGCATTGCCGAGGAGTACCCTCGACAT
-2355           -----+---------+---------+---------+---------+---------+----  -2296

                TCCCCTCCACCAATGGTCTCACATAAATTCAACAAAGCAAACTCATAAAAAGTTTAATGA
-2295           -----+---------+---------+---------+---------+---------+----  -2236

                GTTTCAGAAAGAAATAAAACTAGGCCCCTCCTTTGAGAATCTACGAATGATTCACCATAT
-2235           -----+---------+---------+---------+---------+---------+----  -2176

                CATCTCGCAGTTAGTGATGAGTAACTAAGTCTCAAATTTCCCGACGCATGGCGAAAAAGG
-2175           -----+---------+---------+---------+---------+---------+----  -2116

                TAGCGAACTTAAAATGTGAGGAATGAATGCCACATATGCATGGTGCATCGAGTATTCTCA
-2115           -----+---------+---------+---------+---------+---------+----  -2056

                TTTTAGTCTTGGATTACTCCCTTTAGATGTTGACACCATCCCAAAAATACAACTTGGACA
-2055           -----+---------+---------+---------+---------+---------+----  -1996
```

FIGURE 39 CONTINUED

```
        AGTTGTTCATTTCACTAGTATGAATTTCAGTAAATCGGGCAATACTCCAACACTCATTCA
-1995   -----+----------+----------+----------+----------+----------+----   -1936

        CCCCCTAGGCGAGGTTAGCTCAGATCAACGTCGGGTGTCTTCATCGAGTTAATGTCGTCA
-1935   -----+----------+----------+----------+----------+----------+----   -1876

        CACGCACACACACGTACGCGCACACACACGTGCGCAAACAAAAAGAAAACTAGGAACCTT
-1875   -----+----------+----------+----------+----------+----------+----   -1816

        CTCACGTAGCCTAGGTCTTGTCCTGTAAGAAAAAACCCAGGTCCACCCTAGTTTCGAACC
-1815   -----+----------+----------+----------+----------+----------+----   -1756

        AAAATATTTTTGAAGATACATTAGTAAGATATTTTTGAAAATAAAACCGCAAAAAGGGAA
-1755   -----+----------+----------+----------+----------+----------+----   -1696

        TTGAAAAATATGGACTGGCTGTTTTGTCCAAAACCACATCTTTCGGAGAACCACGAGGGT
-1695   -----+----------+----------+----------+----------+----------+----   -1636

        ATCTATTGATGGGCTCATACTATACCTGGGCATGTGTTGGGCCAGGCCTCATGTCGGGCC
-1635   -----+----------+----------+----------+----------+----------+----   -1576

        GAGGAAAGCCCGACGCTGAAAAATCAGGCCCAAGCTTAACCCGGCCCGACCAAATACCCA
-1575   -----+----------+----------+----------+----------+----------+----   -1516

        CCAAACCCGTTGGGCCATCAGGTTGCGGGCCGGGCAGTAGTGTAAAACACCGATTTCGGG
-1515   -----+----------+----------+----------+----------+----------+----   -1456

        CTACATAGGCCCGGCTCGTTTGTCGGGCAAACATTTCTAGACCTAAGCCCGAGTTTTTCG
-1455   -----+----------+----------+----------+----------+----------+----   -1396

        GGCCGGGCTGCCCATGGCCAGGTATAGCTCATAACGACGTATGACATTTCGAGCAATTGA
-1395   -----+----------+----------+----------+----------+----------+----   -1336

        TGCAAAGCACGTGTAGGGTTTTATCCCATCCGTGTGGCGTGTGTAGGGTGTAAATGAATA
-1335   -----+----------+----------+----------+----------+----------+----   -1276

        GGATAATTTCCTCGCCGAAACTGGTCCCAAATTCGCTTTGAAGTGTCCATATATGATTTT
-1275   -----+----------+----------+----------+----------+----------+----   -1216

        AAAGAATGTGACAAATAAAGATATCCAATTTCGAAATAGTGCTCCGGATACGGTATAGGA
-1215   -----+----------+----------+----------+----------+----------+----   -1156

        TATGGTATAGCAAATAACATGCTGATATGGATTGTCCGATATTAAATTAAGATAATCCAA
-1155   -----+----------+----------+----------+----------+----------+----   -1096

        ATGTTTTAAACCGCATAATTCGATTTTTGAGTCAAAAGCGAATGCCAATTCAGAAGGTTA
-1095   -----+----------+----------+----------+----------+----------+----   -1036

        GCAGTTATTGAGTTTCAAAATTTATTTGGCGAGCATATCTAGTTCTAAATTCTATCACGT
-1035   -----+----------+----------+----------+----------+----------+----   -976

        AAATTGTGTCTTTTTTTAATAACTACACAAGACTAAAAGTTTAAATCTCTCTCAAGATTT
 -975   -----+----------+----------+----------+----------+----------+----   -916

        GCGAAAACTATAGCTATCTACTGATATATATATCCGACTATATTTGTTTTCGGACCGCAT
 -915   -----+----------+----------+----------+----------+----------+----   -856

        GCGTCCTATTTCCGATTCGAATCTGCACTCCGATATATCCACATTGAATCTAAAACCGAT
 -855   -----+----------+----------+----------+----------+----------+----   -796
```

FIGURE 39 CONTINUED

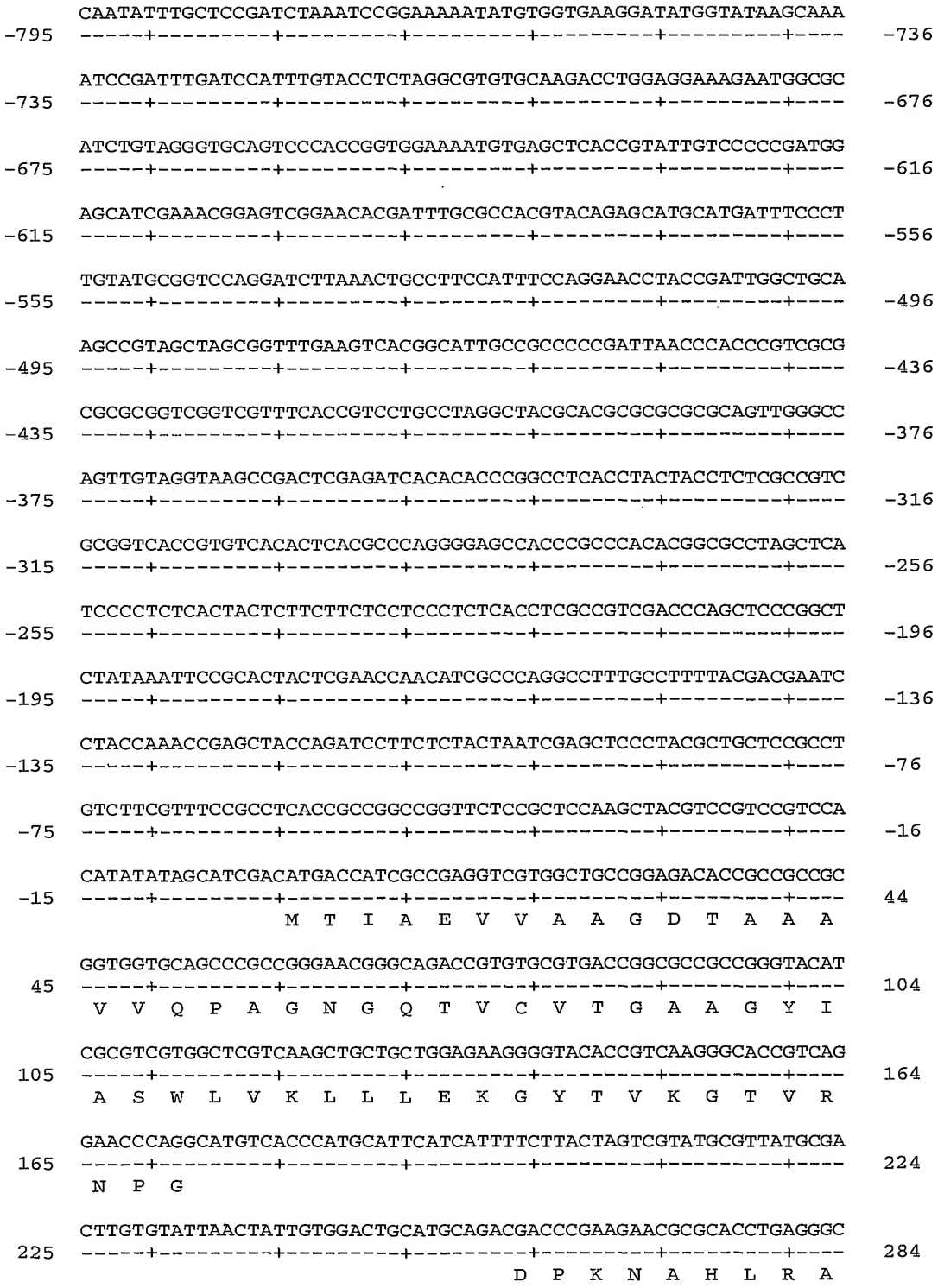


FIGURE 39 CONTINUED

```
                GCTCGACGGCGCCGCCGACCGGCTGGTCCTCTGCAAGGCCGACCTCCTCGACTACGACGC
285             -----+---------+---------+---------+---------+---------+----  344
                 L  D  G  A  A  D  R  L  V  L  C  K  A  D  L  L  D  Y  D  A

                CATCCGCCGCGCCATCGACGGCTGCCACGGCGTCTTCCACACCGCGTCCCCCGTCACCGA
345             -----+---------+---------+---------+---------+---------+----  404
                 I  R  R  A  I  D  G  C  H  G  V  F  H  T  A  S  P  V  T  D

                CGACCCCGTACGTACTCCATAGAACTCGGCACCCCTAGCTTCTCTCCGTTCTCTCTGTAT
405             -----+---------+---------+---------+---------+---------+----  464
                 D  P

                GTCTGTCACCGTCGATCGCCATGGCAGCACGCATGCATGCGCGCGCAACGCTAGCTAGAC
465             -----+---------+---------+---------+---------+---------+----  524

                GCTGACCGACTCATTGTGCAGGAGCAAATGGTGGAGCCGGCGGTGAGGGGCACGCAGTAC
525             -----+---------+---------+---------+---------+---------+----  584
                                   E  Q  M  V  E  P  A  V  R  G  T  Q  Y

                GTCATAGACGCGGCGGCGGAGGCCGGCACGGTGCGGCGGATGGTGCTCACCTCCTCCATC
585             -----+---------+---------+---------+---------+---------+----  644
                V  I  D  A  A  A  E  A  G  T  V  R  R  M  V  L  T  S  S  I

                GGCGCCGTCACCATGGACCCCAACCGCGGGCCGGACGTGGTCGTCGACGAGTCGTGCTGG
645             -----+---------+---------+---------+---------+---------+----  704
                G  A  V  T  M  D  P  N  R  G  P  D  V  V  V  D  E  S  C  W

                AGCGACCTCGACTTCTGCAAGAAAACCAGGGTGGGTGCTGCATGCTCAATTTTTATTATC
705             -----+---------+---------+---------+---------+---------+----  764
                S  D  L  D  F  C  K  K  T  R

                ATAGCTACCCTTTTTCTGCACCATGCTGCATTTCTTTTCCAAAAACAACTCTCAAAAGAT
765             -----+---------+---------+---------+---------+---------+----  824

                ATGCTACGTGGTGAGTTCCTATAGCTGAATTATTACAACTACCACCCTATCGATCACTAC
825             -----+---------+---------+---------+---------+---------+----  884

                CGCCCTAAAAGTGTTCAACTTTTGAAGGCAACCAAAACCAATACATGAACGACGATCGTG
885             -----+---------+---------+---------+---------+---------+----  944

                TGCGCTTGTCGTCGTTATCATTAGCCTCTGTAGCTCTAATTTTCACCTATGTACGCATGG
945             -----+---------+---------+---------+---------+---------+----  1004

                ATAGACGATTCGGAAATACAGTTCAGTTTACCTACCATATACTATGCCGAAATCGAACGC
1005            -----+---------+---------+---------+---------+---------+----  1064

                ACACAGGTGTGAGGCAGCAGCCGCTCACGAGTTATGCGCCGAAACCGACATCTCGGAATC
1065            -----+---------+---------+---------+---------+---------+----  1124

                TTCAGTCCACAATCAAAAAATAGACACCTGGTACCACTACAAAATTATACTCCTACTGTA
1125            -----+---------+---------+---------+---------+---------+----  1184

                TATTGGTAAAACAAAACATTTTCTTTTTTATTTGATAGGAGTGCTGCAAATTAAAGTTCT
1185            -----+---------+---------+---------+---------+---------+----  1244

                TTGTGTCATTTTTCAAAGGAAAAAAAAAACACCTTTACCACTCTTCTTCCTTGCCATCAT
1245            -----+---------+---------+---------+---------+---------+----  1304
```

FIGURE 39 CONTINUED

```
      TTTTTTTTTACCAAAGTTTGTTCTGTCAAATGAACATATATATAGTTCGGTGCTATGTCA
1305  -----+----------+---------+---------+---------+---------+----  1364

      GTGCCATTTACCGGCCACTAGCTAGTAGGACTGCCATGTTCCAGCAAATTGTCTAGTGGA
1365  -----+---------+---------+---------+---------+---------+----  1424

      CCGGAGTGGCCAAAAGGAGCCAATTATGTAGGGTTGCAAGCGGGATCACACAAAAGCCTC
1425  -----+---------+---------+---------+---------+---------+----  1484

      GCCTCTAGTTCATTTTATCAATTAAGTGGTACTTTCTCAGGGACCCCCCTTGCAACTCTA
1485  -----+---------+---------+---------+---------+---------+----  1544

      CCATTACATCCGTGCAAAATAAAAGCTAGCATCACGCACCAGATTTAGTACTCCCTCCGT
1545  -----+---------+---------+---------+---------+---------+----  1604

      TTTTATTTAGTTCGCATTCTAGGTTCAGCCAAAGTCATACTTTGCAAAGTTTAACCAAAA
1605  -----+---------+---------+---------+---------+---------+----  1664

      TTATAAGAAAAAAATATCAATAATCATCATACAAAATACATATAATATAAGAGTAAACCT
1665  -----+---------+---------+---------+---------+---------+----  1724

      TATAACGATTCTACAATAGATTTTTTTATTGCATATGTCAATATTTTTTCATAAATATTT
1725  -----+---------+---------+---------+---------+---------+----  1784

      ACTCAAAATTATAAGGTTTGACTTTGACTAAACCCAGAACCTTCTTAGAGAGGAAGAAAT
1785  -----+---------+---------+---------+---------+---------+----  1844

      GCATGGGCAAAAGCAAATCATGCATATGGGCAGGAGTAACATTTTTTTGACTTTCATAGA
1845  -----+---------+---------+---------+---------+---------+----  1904

      AAGTACTGTATGGCACTAAACGGTCTAAACCGGACACTGGAAGCAAATCGTGCACGTGGG
1905  -----+---------+---------+---------+---------+---------+----  1964

      CAATATTATCTACCGTCGCGTCGCCAGTCTCCCCATGCCCATGACCATGCTTGGAATTTT
1965  -----+---------+---------+---------+---------+---------+----  2024

      AGTCTCGCCGGAGCTGCCGAGTGCATGCATAGTGACGAGTTTCAATAGGCCACTATATAT
2025  -----+---------+---------+---------+---------+---------+----  2084

      GTGATCATGGCTCTTGATTTGTCACTTTCTTTTTTTGCCGAAGGATATAGTAGTATTACT
2085  -----+---------+---------+---------+---------+---------+----  2144

      TTCTCTGCTATCACAAAGAAAGAACTGATTGTGTCTAGTCTAGGTGGTCTCAGAATTCTG
2145  -----+---------+---------+---------+---------+---------+----  2204

      CATGACTCCAGAGTATTCTTGATGCCACTTGTTTGTTATTGCAAGAAACTTAATTCGGAG
2205  -----+---------+---------+---------+---------+---------+----  2264

      ACAACCAAAAGCTCATCCCATGTCTCTGGAACTAGTAGACATAAGAAAATCTCATGGTAT
2265  -----+---------+---------+---------+---------+---------+----  2324

      CAGTTTGCTATTTATCTACAACTGAAACGGCATGTTTGGTTTTATTAAATTCAGAACTGG
2325  -----+---------+---------+---------+---------+---------+----  2384
                                                        N   W

      TACTGCTACGGGAAGGCGGTTGCGGAGCAGGCGGCATCGGAGTTGGCGCGGCAGCGCGGC
2385  -----+---------+---------+---------+---------+---------+----  2444
      Y   C   Y   G   K   A   V   A   E   Q   A   A   S   E   L   A   R   Q   R   G
```

FIGURE 39 CONTINUED

```
      GTGGACCTTGTGGTGGTGAACCCGGTGCTGGTGATCGGCCCCCTGCTGCAGCCGACGGTG
2445  -----+---------+---------+---------+---------+---------+----  2504
      V  D  L  V  V  V  N  P  V  L  V  I  G  P  L  L  Q  P  T  V

      AACGCCAGCATCGGCCACATCCTCAAGTACCTGGACGGGTCGGCCAGCAAGTTCGCCAAC
2505  -----+---------+---------+---------+---------+---------+----  2564
      N  A  S  I  G  H  I  L  K  Y  L  D  G  S  A  S  K  F  A  N

      GCCGTGCAGGCGTACGTGGACGTCCGCGACGTGGCCGACGCCCACCTCCGCGTCTTCGAG
2565  -----+---------+---------+---------+---------+---------+----  2624
      A  V  Q  A  Y  V  D  V  R  D  V  A  D  A  H  L  R  V  F  E

      TGCGCCGCCGCGTCCGGCCGCCACCTCTGCGCCGAGCGCGTCCTCCACCGCGAGGACGTC
2625  -----+---------+---------+---------+---------+---------+----  2684
      C  A  A  A  S  G  R  H  L  C  A  E  R  V  L  H  R  E  D  V

      GTGCGCATCCTCGCCAAGCTCTTCCCCGAGTACCCCGTCCCCACCAGGTACGCGTACGAC
2685  -----+---------+---------+---------+---------+---------+----  2744
      V  R  I  L  A  K  L  F  P  E  Y  P  V  P  T  R

      CTGCTTGCTAGCCGCTTCCGTTAATTCCATTGCCTTAATTGATTGCATGATGCCGCTCCT
2745  -----+---------+---------+---------+---------+---------+----  2804

      AATTTACTCACTTGCGTAACTAATTGCATTCATATATGATCTACCAACCGTGGAGAAAAT
2805  -----+---------+---------+---------+---------+---------+----  2864

      TAGCAAGAGTCTGTCGGGGCGTCCCGGTCCAGTGCAGTTAACCTGCATGTCGATGGTCTG
2865  -----+---------+---------+---------+---------+---------+----  2924

      CAGGTTGCAGCTTACTTGTGGTTCTTTAGTTCAGAGACACAGAGCAATTGGGCACTAAGC
2925  -----+---------+---------+---------+---------+---------+----  2984

      AAAACTGACATCACTGGTAATTAGGTAGCTCCCACACACTGAAGTGGGTGGATCCCATCG
2985  -----+---------+---------+---------+---------+---------+----  3044

      GTAGTAGGTAAGGGTGGATAGTACTGGACGAGAGCTCGATCGTTGTTGTAAAAAAGCGAG
3045  -----+---------+---------+---------+---------+---------+----  3104

      TGACCACCACTTCACCATCCACTGCAAGTAGCTGCTAGTGAACCATCCAACCAGCTCCCT
3105  -----+---------+---------+---------+---------+---------+----  3164

      GGATCACTCTGCTCCGTCCGTACCTTCAGCTACCTACAGAAGCGACATGAACACACAGAC
3165  -----+---------+---------+---------+---------+---------+----  3224

      ACACAAGGCCGGCTCACCATTCGCATAGGTCAAACCAAATGTTGGTGAACGGCAACATCG
3225  -----+---------+---------+---------+---------+---------+----  3284

      CCACAAGTCGCGTGCTAGTTCGAGGTTGTGTCCGGTGTACCGAGGCCACACTATTCGTGC
3285  -----+---------+---------+---------+---------+---------+----  3344

      TGCCCGTCGCTGATATTTGCACGCGTAGCTGTCGACGAAAGTAGGTGGACTGACAGATAC
3345  -----+---------+---------+---------+---------+---------+----  3404

      ACATATCCTCATTGCCTTCTCTGCTCGGTTTCTGCTAGGATTGCCATCTTCAGGAGTGCC
3405  -----+---------+---------+---------+---------+---------+----  3464

      TATCCGCACGGCAGAAACGCGTAGCATCAGGCCAGAAAGCAGCGTGCGTGATATCGTAAC
3465  -----+---------+---------+---------+---------+---------+----  3524
```

FIGURE 39 CONTINUED

```
                CCAGACGGTCTTCACCTGTCCATTCTGGGCTACCTGGCATACTACCTCGGTGCCGCTGTG
3525  ------+---------+---------+---------+---------+---------+---------+----  3584

                CCGCTGACCAATTCGTGCACGACCACTATAGCAAAACCCTATGCATGTAACTGCTTCAAG
3585  ------+---------+---------+---------+---------+---------+---------+----  3644

                ATCAGCAGTGACATGTGCAATATAAACCTCAAGTGTGCACTCTAGTGCGTACTGATAAAA
3645  ------+---------+---------+---------+---------+---------+---------+----  3704

                CCGTATAACTGGTGACCCAGTCATTCTTCTCTTTTTTATTTGTTTGGACCAAACGAACAC
3705  ------+---------+---------+---------+---------+---------+---------+----  3764

                AGCATGTTATCCATCACCAACAAGTGGCGCTGATTTTTCAAACTACACTGGGATCATACT
3765  ------+---------+---------+---------+---------+---------+---------+----  3824

                GGAAACCAAAGCAGGAGAACATCTTCGAACCAAGAGATGTTTACTAAATTTGAAAGAAAA
3825  ------+---------+---------+---------+---------+---------+---------+----  3884

                TGTACTGACAAGTAATCTGTCTGAAGCAAGACACATACTACCTCGGTTCGAACGTGGGAC
3885  ------+---------+---------+---------+---------+---------+---------+----  3944

                ACCATGCCCGTGCCATATTTGCTAGGCACCACTCTGCCGTCGATTGTATCCCAACGGAGG
3945  ------+---------+---------+---------+---------+---------+---------+----  4004

                GAGTATCGATTTGCGCAAAGTTCCTACATACATAGCCGCTCAAGATATAATCTTACGACC
4005  ------+---------+---------+---------+---------+---------+---------+----  4064

                TTCCGTCGAAATCGGTGATACGTCGCAACCTATAGCTAACTTGGCAGAGCATAAAATAAC
4065  ------+---------+---------+---------+---------+---------+---------+----  4124

                TATCTAAGGTTGGGGGTCTCCCTCTTTTCAATCAACCTTTCATACCGAATGATGGGAGTGT
4125  ------+---------+---------+---------+---------+---------+---------+----  4184

                TTGTGAAAACATCTCTTGGTCGACTCAGCATTAGCGCCCTACCAATTTCTCTGTGGACAA
4185  ------+---------+---------+---------+---------+---------+---------+----  4244

                TGCCACCTTAAATCGTTTTTTAGTCTTCATGATTTACTCCCCCTTATATCTGGCCGTAGT
4245  ------+---------+---------+---------+---------+---------+---------+----  4304

                CCCTCTTTTCCATTTTTCTTGTCTGGTTTTAAGTCAAATTTAGACTACTAAAACAACAGC
4305  ------+---------+---------+---------+---------+---------+---------+----  4364

                AAGATTTTATGGAAGGGAGGTAGTGCAAAACAGAAAGTCCGATCGAAATGCGTGCCAATT
4365  ------+---------+---------+---------+---------+---------+---------+----  4424

                TGTCGTCGCGGCGGCCGGACTAAAATGGATCTGCATGTGCATACCGTTCGTCGGAGTATC
4425  ------+---------+---------+---------+---------+---------+---------+----  4484

                CTGCGAACGGTCGTGTGTTTAGTCAACATTAATGTGAGGTTCATGTGATACTCTTGCTTG
4485  ------+---------+---------+---------+---------+---------+---------+----  4544

                AAAGATACTACTACTGCTACCTCGTAGAACTGAATGAAAGTATGTGGGACTGTTCAGCTC
4545  ------+---------+---------+---------+---------+---------+---------+----  4604

                TCTGCACATGTCAAATGTCGTTACTCATACCTTTCGTCAGAGCATCCTGCGACGCGCGCC
4605  ------+---------+---------+---------+---------+---------+---------+----  4664

                GGTGCCGAAATTTCGCCGTGTGTTTAGTCAAGATCAACGTGAGGTTCATGCGGTACCCTA
4665  ------+---------+---------+---------+---------+---------+---------+----  4724
```

FIGURE 39 CONTINUED

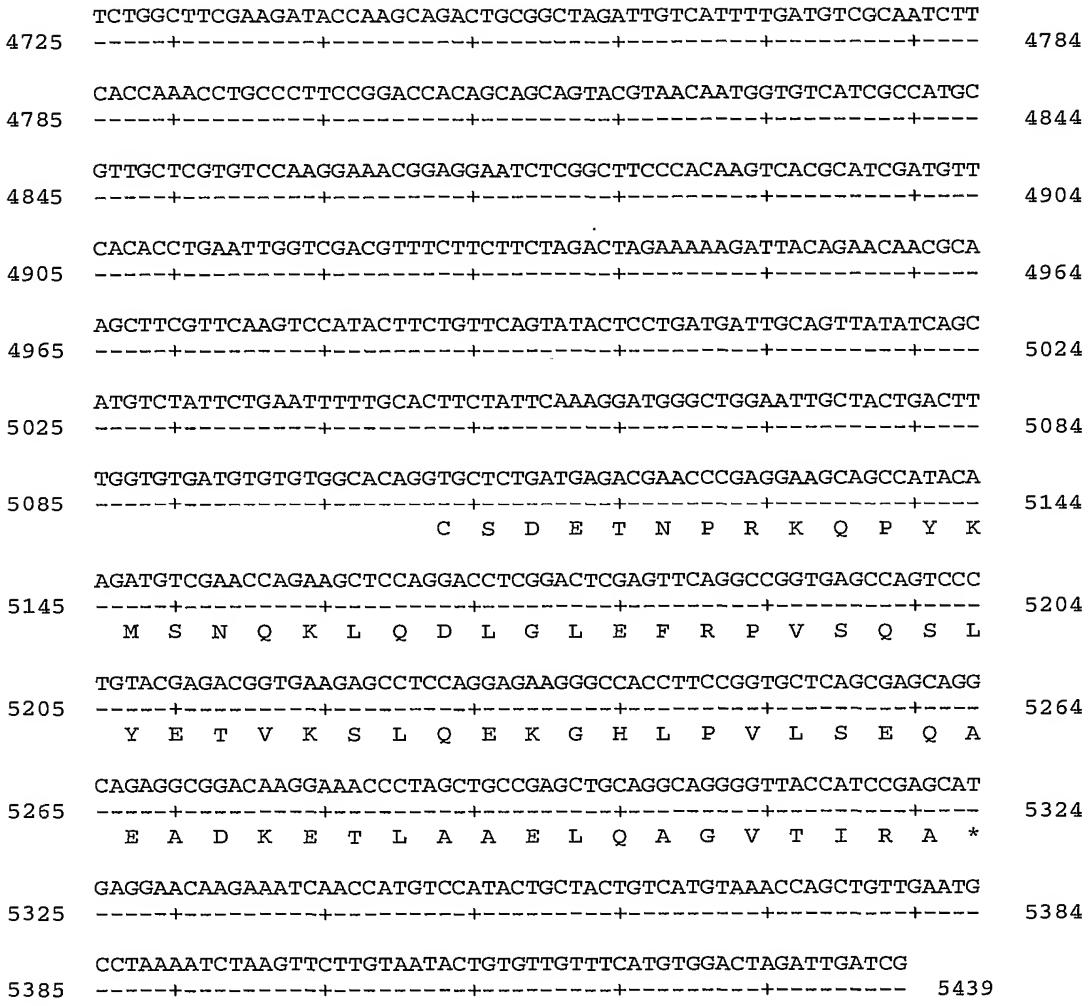
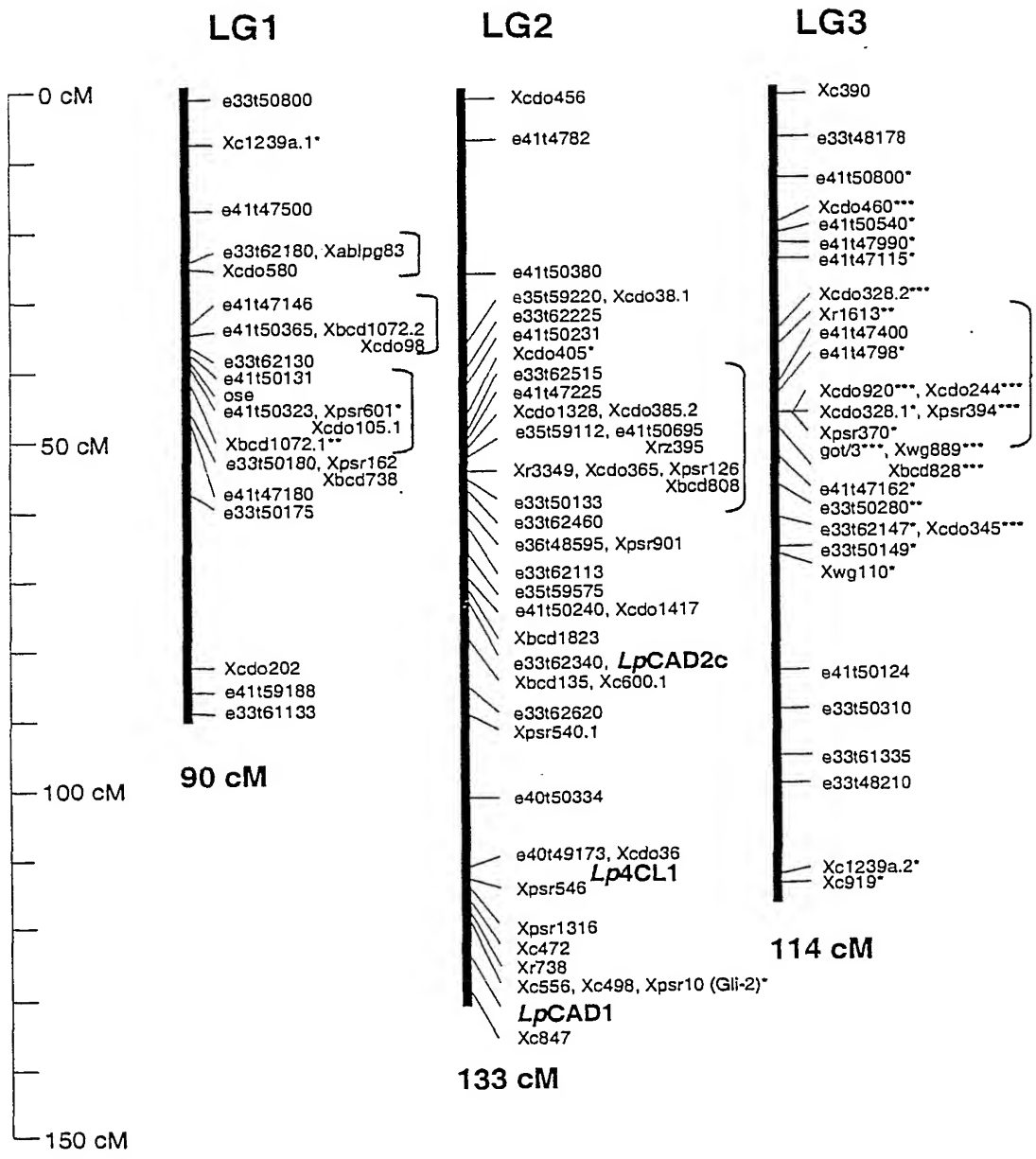


FIGURE 39 CONTINUED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Xr738
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LpCAD1
Xc847
133 cM
Xc390
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e41t50800*
Xcdo460***
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Xcdo328.2***
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FIGURE 40

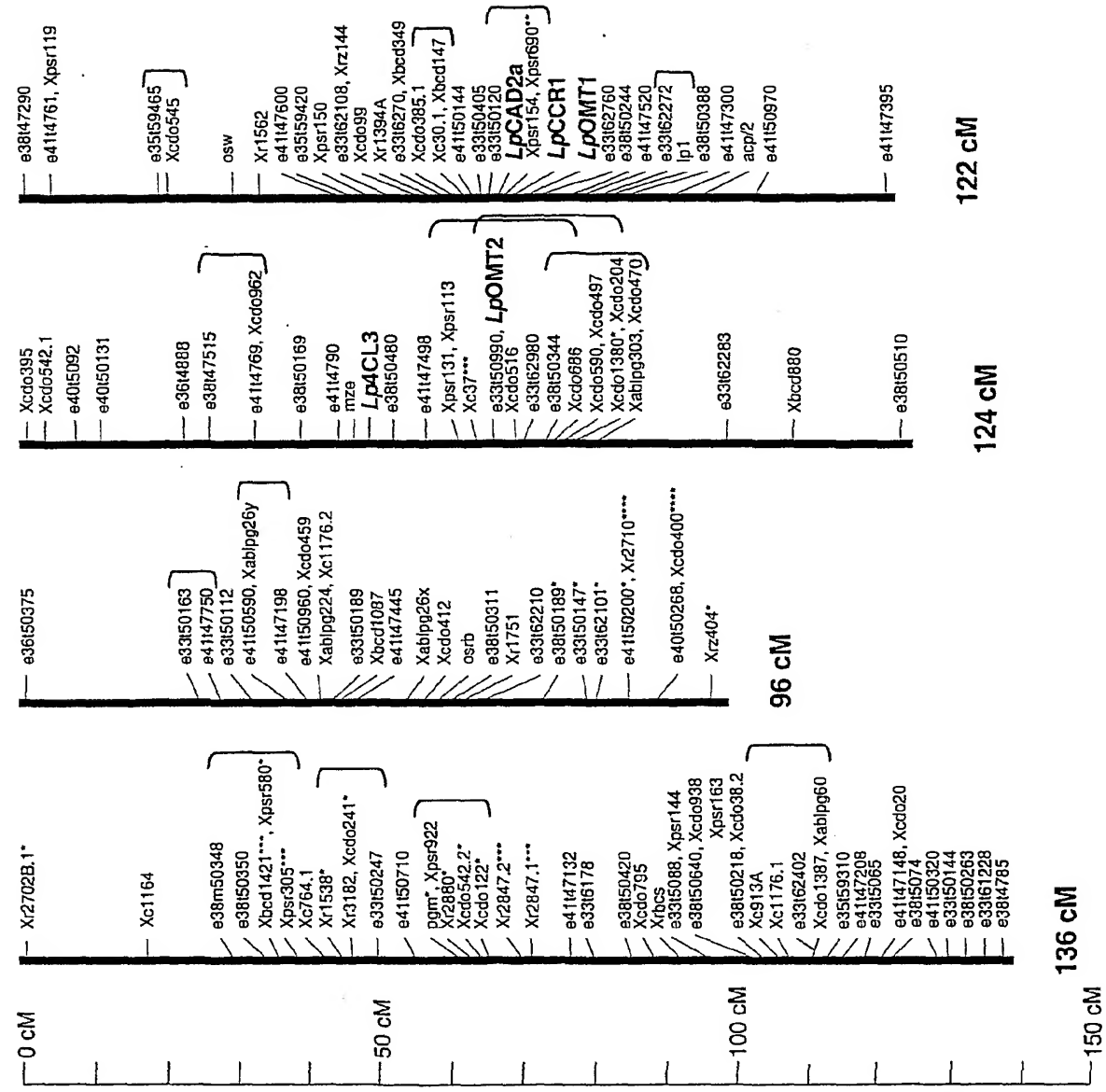

FIGURE 40 CONTINUED

Organization Applicant

Street : 15th Floor, 8 Nicholson Street
City : East Melbourne
State : Victoria
Country : Australia
PostalCode : 3002
PhoneNumber :
FaxNumber :
EmailAddress :
<110> OrganizationName : State of Victoria as represented by Department of Natural Resources and Environment

Organization Applicant

Street : North Terrace
City : Adelaide
State : South Australia
Country : Australia
PostalCode : 5005
PhoneNumber :
FaxNumber :
EmailAddress :
<110> OrganizationName : The University of Adelaide

Organization Applicant

Street : Lisboa 27, Apartado Postal 6-641
City : Mexcio
State : DF
Country : Mexico
PostalCode : 06600
PhoneNumber :
FaxNumber :
EmailAddress :
<110> OrganizationName : International Maize and Wheat Improvement Center

Organization Applicant

Street : Waite Road
City : Glen Osmond
State : South Australia
Country : Australia
PostalCode : 5064
PhoneNumber :
FaxNumber :
EmailAddress :
<110> OrganizationName : State of South Australia as represented by South Australian Research and Development Institute

Organization Applicant

Street : Military Road
City : Lismore
State : New South Wales
Country : Australia
PostalCode : 2580
PhoneNumber :
FaxNumber :
EmailAddress :
<110> OrganizationName : Southern Cross University

Organization Applicant

Street : Level 3, 84 William Street
City : Melbourne
State : Victoria
Country : Australia

PostalCode : 3000
PhoneNumber :
FaxNumber :
EmailAddress :
<110> OrganizationName : Dairy Research and Development Corporation

Application Project

<120> Title : Modification of Lignin Biosynthesis
<130> AppFileReference : 40494788
<140> CurrentAppNumber : AU PQ8154
<141> CurrentFilingDate : 2001-06-14

Earlier Applications

<150> PriorAppNumber : AU PQ8154
<151> PriorFilingDate : 2000-06-14

Sequence

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<211> Length : 2284
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SequenceDescription :

Custom Codon

Sequence Name : 4CL1cDNA (SEQ ID NO: 1)

Sequence

<213> OrganismName : Lolium perenne
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SPDDA VALPYSSGTT GLPKGVVLTH GGLVSSVAQQ 240VDGENPNLHM RAGEDVVLCV LPLFHIFSLN SVL
LCALRAG AAVMLMPRFE MGAMLEGIER 300WRVTVAAVVP PLVLALAKNP GVEKHDLSSI RIVLSGAAPL G
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<212> Type : PRT
<211> Length : 606
SequenceName : 4CL1pep (SEQ ID NO: 2)
SequenceDescription :

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :
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ttc agggagatca aaaaaaaaaa 1980aaaaaaaaaa aa
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<212> Type : DNA
<211> Length : 1992
SequenceName : 4CL2cDNA (SEQ ID NO: 3)
SequenceDescription :

Custom Codon

Sequence Name : 4CL2cDNA (SEQ ID NO: 3)

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :
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A GLRRLGVGKG DVVMALLRNC PEFAFVFLGA ARLGAATTTA NPFYTPHEIH 120RQATAAGARV IVTEACA
VEK VRAFAAERGI PVVSVDEGVD GGCLPFAETL LGEESGERFV 180DEAVDPDDVV ALPYSSGTTG LPKGV
MLTHR SLVTSVAQQV DGENPNLHFS SSDVLLCVLP 240LFHIYSLNSV LLAGLRAGCA IVIMRKFDHG ALV
DLVRTHG VTVAPFVPPI VVEIAKSARV 300TAADLASIRL VMSGAAPMGK ELQDAFMAKI PNAVLGQGYG M
TEAGPVLAM CLAFAKEPFA 360VKSGSCGTVV RNAELKIVDP DTGASLGRNL PGEICIRGKQ IMKGYLNDPV
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EL 480AGEVPVAFVV RTEGSEISEN EIKQFVAKEV VFYKRICKVF FADSIPKSPS GKILRKDLRA 54
0KLAAGIPSSN TTQSKS 556

<212> Type : PRT
<211> Length : 556
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SequenceDescription :

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :
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ccttcctggg 420cgcggcgcgg ctgggcgccg ccaccaccac cgccaacccg ttctacaccc cgcacgag
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gc gtggacttcg cggagctgat cgccggcgag gagctgcccg aggcggacga 660ggccggggtc ctcccc
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<212> Type : DNA
<211> Length : 2038
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SequenceDescription :

Custom Codon

Sequence Name : 4CLcDNA (SEQ ID NO: 5)

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :
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R RAAAGLRRMG VGKGDVVMNL LRNCPEFAFS FLGAARLGAA TTTANPFYTP 120HEIHRQAEAA GAKLIVT
EAC AVEKVLEFAA GRGVPVVTVD GRRDGCVDFA ELIAGEELPE 180ADEAGVLPDD VVALPYSSGT TGLPK
GVMLT HRSLVTSVAQ LVDGSNPNVC FNKDDALLCL 240LPLFHIYSLH TVLLAGLRVG AAIVIMRKFD VGA
LVDLVRA HRITIAPFVP PIVVEIAKSD 300RVGADDLASI RMVLSGAAPM GKDLQDAFMA KIPNAVLGQG Y
GMTEAGPVL AMCLAFAKEP 360FKVKSGSCGT VVRNAELKVV DPDTGASLGR NQPGEICVRG KQIMIGYLND

PESTKNTIDK 420DGWLHTGDIG LVDDDDEIFI VDRLKEIIKY KGFQVAPAEL EALLLTNPEV KDAAVVGV
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SequenceName : 4CLpep (SEQ ID NO: 6)
SequenceDescription :

Sequence

<213> OrganismName : Lolium perenne
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<212> Type : DNA
<211> Length : 1395
SequenceName : CCR1cDNA (SEQ ID NO: 7)
SequenceDescription :

Custom Codon

Sequence Name : CCR1cDNA (SEQ ID NO: 7)

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :
MTIAEVVAAG DTAAAVVQPA GNGQTVCVTG AAGYIASWLV KLLLEKGYTV KGTVRNPDDP 60KNAHLRALD
G AADRLVLCKA DLLDYDAIRR AIDGCHGVFH TASPVTDDPE QMVEPAVRGT 120QYVIDAAAEA GTVRRMV
LTS SIGAVTMDPN RGPDVVVDES CWSDLDFCKK TRNWYCYGKA 180VAEQAASELA RQRGVDLVVV NPVLV
IGPLL QPTVNASIGH ILKYLDGSAS KFANAVQAYV 240DVRDVADAHL RVFECAAASG RHLCAERVLH RED
VVRILAK LFPEYPVPTR CSDETNPRKQ 300PYKMSNQKLQ DLGLEFRPVS QSLYETVKSL QEKGHLPVLS E
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<211> Length : 362
SequenceName : CCR1pep (SEQ ID NO: 8)
SequenceDescription :

Sequence

<213> OrganismName : Lolium perenne
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<212> Type : DNA
<211> Length : 1325
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SequenceDescription :

Custom Codon

Sequence Name : CAD1cDNA (SEQ ID NO: 9)

Sequence

<213> OrganismName : Lo1ium perenne
<400> PreSequenceString :
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G VVASVGSGVS SFKAGDTVGV GYFLDSCRTC YSCSKGYENF CPTLTLTSNG 120VDGGGATTQG GFSDVLV
VNK DYVIRVPDNL PLAGAAPLLC AGVTVYSPMV EYGLNAPGKH 180XGVVGLGGLG HVAVKFGKAF GMTVT
VISSS DRKRDEALGR LGADAFLVSS DPEQMKAAAG 240TMDGIIDTVS AGHPIVPLLD LLKPMGQMVV VGA
PSKPLEL PAFAIIGGGK RLAGSGTGSV 300AHCQAMLDFA GKHGITADVE VVKMDYGQHR HRAAREERRQ V
PLRHRRRRQ PPAGHRRLTC 360ATQCGRALVW SRKRFAGSQP HEQVNESLVC CLSSFHIWDA VPDFHVK
407

<212> Type : PRT
<211> Length : 407
SequenceName : CAD1pep (SEQ ID NO: 10)
SequenceDescription :

Sequence

<213> OrganismName : Lo1ium prenne
<400> PreSequenceString :
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cag cacaccagga aggcggtggg gctggcggcg cgcgacgacg 180ccggccacct ctccccgctc gccat
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1320tgtttgcgag gaaactgagt tgaaggatgg atggataaaa aaaaaaaaaa aaaaaaa 1377

<212> Type : DNA
<211> Length : 1377

SequenceName : CAD2cvEllettcDNA (SEQ ID NO: 11)
SequenceDescription :

Custom Codon

Sequence Name : CAD2cvEllettcDNA (SEQ ID NO: 11)

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :
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R YPMIPGHEIA GEVTEVGKNV SKFKAGDRVG VGCMVNSCRS CESCDKGFEN 120HCPGMILTYN SVDVDGT
VTY GGYSSMVVVH ERFVVRFPDA MPLDKGAPLL CAGITVYSPM 180KYHGLNVPGL HLGVLGLGGL GHVAV
KFGKA FGMKVTVISS SPGKKEEALG RLGADAFIVS 240KDADEMKAVI APWMASXNTV SANIPLTPLF GLL
KPNGKMI MVGLPEKPIE IPPFALVATN 300KTLAGSIIGG MSDTQEMLDL AAKHGVTADI EVVGAEYVNT A
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370
<212> Type : PRT
<211> Length : 370
SequenceName : CADcvE11ettepep (SEQ ID NO: 12)
SequenceDescription :

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :
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5119

<212> Type : DNA
<211> Length : 5119
SequenceName : LpOmt1promoter (SEQ ID NO: 13)
SequenceDescription :

Custom Codon

Sequence Name : LpOmt1promoter (SEQ ID NO: 13)

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :
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<212> Type : DNA
<211> Length : 4555
SequenceName : CAD2cvBar1anogenomic (SEQ ID NO: 14)
SequenceDescription :

Custom Codon

Sequence Name : CAD2cvBar1anogenomic (SEQ ID NO: 14)

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :

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TVY SPMKYHGLNV PGLHLGVLGL GGLGHVAVKF GKAFGMKVTV 180ISSSPGKKEE ALGRLGADAF IVSKD
ADEMK AVMSTMDGII NTVSANIPLT PLFGLLFALV 240ANKTLAGSII GGMSDTQEML DLAAKHGVTA DIE
VVGAEYV NTALERLAKN DVRYRFVIDI 300GNTLDKVAAT TE
312

<212> Type : PRT
<211> Length : 312
SequenceName : CAD2cvBar1anopep (SEQ ID NO: 15)
SequenceDescription :

Sequence

<213> OrganismName : Lolium perenne
<400> PreSequenceString :
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00699

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A01H 5/00, C12N 9/00, C12N 9/02, C12N 9/04, C12N 9/10, C12N 15/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

GenBank, EMBL, PDB Nucleic Acids, GenPept, TREMBL, SWISS-PROT, PIR, Medline, ChemAbs, AGRICOLA, WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 952 486 A (BLOKSBERG *et al*) 14 September 1999 whole of document	1-26
X	Civardi L *et al*, "Molecular Cloning and Characterization of two cDNAs Encoding Enzymes Required for Secondary Cell Wall Biosynthesis in Maize", *NATO ASI Series, Volume H 104 (Cellular Integration of Signalling Pathways in Plant Development)*, 1998, pages 135-146 whole of document	1, 2, 4-12, 14-17, 20a, 19b, 23-26
X	WO 99/31243 A (INTERNATIONAL PAPER COMPANY) 24 June 1999 whole of document	1-3, 6-13, 16, 17, 19a, 19b, 21, 23-26

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 July 2001	29 August 2001
Name and mailing address of the ISA/AU	Authorised officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**GARETH COOK** Telephone No : (02) 6283 2541

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00699

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GenBank accession AF052223, Heath RL *et al*, "Lolium perenne 4-coumarate-CoA ligase 4CL3 mRNA, complete cds", 7 March 2000.	1-3, 6-13, 16, 17, 19a, 19b, 21, 23-26
X	GenPept accession AAF37734, Heath RL *et al*, "4-coumarate-CoA ligase 4CL3 [Lolium perenne]", 7 March 2000.	1-3, 6-13, 16, 17, 19a, 19b, 21, 23-26
X	GenBank accession AF052222, Heath RL *et al*, "Lolium perenne 4-coumarate-CoA ligase 4CL2 mRNA, complete cds", 7 March 2000.	1-3, 6-13, 16, 17, 19a, 19b, 21, 23-26
X	GenPept accession AAF37733, Heath RL *et al*, "4-coumarate-CoA ligase 4CL2 [Lolium perenne]", 7 March 2000.	1-3, 6-13, 16, 17, 19a, 19b, 21, 23-26
X	GenBank accession AF052221, Heath RL *et al*, "Lolium perenne 4-coumarate-CoA ligase 4CL1 mRNA, complete cds", 7 March 2000.	1-3, 6-13, 16, 17, 19a, 19b, 21, 23-26
X	GenPept accession AAF37732, Heath RL *et al*, "4-coumarate-CoA ligase 4CL1 [Lolium perenne]", 7 March 2000.	1-3, 6-13, 16, 17, 19a, 19b, 21, 23-26
X	WO 98/39454 A (ZENECA LIMITED) 11 September 1998 whole of document	1, 2, 4, 6-12, 14, 17, 20a, 19b, 22-26
X	Pichon M *et al*, "Cloning and characterization of two maize cDNAs encoding Cinnamoyl-CoA Reductase (CCR) and differential expression of the corresponding genes", *Plant Molecular Biology*, 1998, 38:671-676 whole of document	1, 2, 4, 6-12, 14, 17, 20a, 19b, 22-26
X	GenBank accession AJ231134, Selman-Housein G *et al*, "Saccharum officinarum mRNA for cinnamoyl-CoA reductase" 25 January 2000	1, 2, 4, 6-12, 14, 17, 20a, 19b, 22-26
X	WO 93/05159 A (IMPERIAL CHEMICAL INDUSTRIES PLC) 18 March 1993 whole of document	1, 2, 5-12, 15-17, 19b, 23-26
X	WO 93/24638 A (ZENECA LIMITED) 9 December 1993 whole of document	1, 2, 5-12, 15-17, 19b, 23-26
X	Baucher M *et al*, "Down-regulation of cinnamyl alcohol dehydrogenase in transgenic alfalfa (*Medicago sativa* L.) and the effect on lignin composition and digestibility", *Plant Molecular Biology*, 1999, 39:437-447 whole of document	1, 2, 5-12, 15-17, 19b, 23-26
X	GenBank accession AF010290, McAlister FM *et al*, "Lolium perenne cinnamyl alcohol dehydrogenase mRNA, complete cds", 23 September 1997	1, 2, 5-12, 15-17, 19b, 23-26
X	GenPept accession AAB70908, McAlister FM, "cinnamyl alcohol dehydrogenase [Lolium perenne]", 22 September 1997	1, 2, 5-12, 15-17, 19b, 23-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00699

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Heath R *et al*, "cDNA Cloning and Differential Expression of Three Caffeic Acid *O*-Methyltransferase Homologues from Perennial Ryegrass (*Lolium perenne*)," *Journal of Plant Physiology*, 1998, 153:649-657 whole of document	17, 18, 19b, 20b, 23-26
X	GenBank accession AF033540, Heath RL et al, "Lolium perenne caffeic acid O-methyltransferase (OMT3) mRNA, complete cds", 29 January 1999	17, 18, 19b, 20b, 23-26
X	GenBank accession AF033539, Heath RL et al, "Lolium perenne caffeic acid O-methyltransferase (OMT2) mRNA, complete cds", 29 January 1999	17, 18, 19b, 20b, 23-26
X	GenBank accession AF033548, Heath RL et al, "Lolium perenne caffeic acid O-methyltransferase (OMT1) mRNA, complete cds", 29 January 1999	17, 18, 19b, 20b, 23-26
X	GenBank accession AF010291, McAlister FM *et al*, "Lolium perenne bispecific caffeic acid/hydroxyferulic acid O-methyltransferase mRNA, complete cds", 3 June 1998	17, 18, 19b, 20b, 23-26
X	Capellades M *et al*, "The maize caffeic acid *O*-methyltransferase gene promoter is active in transgenic tobacco and maize plants," *Plant Molecular Biology*, 1996, 31:307-322 whole of document	17, 18, 19b, 20b, 23-26
	Note that the application as filed contains two claims numbered 19 and two claims numbered 20. To distinguish them, 19a and 20a refer to the first claims 19 and 20, and 19b and 20b refer to the second claims 19 and 20.	

INTERNATIONAL SEARCH REPORT

International application No.
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Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: II, Observations where unity of invention is lacking

The international search report has been drawn up in respect of the entire international application but the International Searching Authority is of the opinion that the application does not appear to comply with the requirements of unity of invention as set forth in the PCT regulations (Article 34(3), Rule 68(1) PCT).

The separate groups of invention are:

1. Claims 1, 2, 6 to 12, 16, 17,19a, 19b and 23 to 26 (partial) and claims 3, 13 and 21 (complete) are to 4-coumarate-CoA ligase (4CL) from ryegrass (*Lolium*), the nucleotide sequence encoding it, the promoter from its gene and various uses of them. 4CL from ryegrass is considered to be the first "special technical feature".

2. Claims 1, 6 to 11, 16, 17, 19a, 19b and 23 to 26 (partial) are to 4CL from fescue (*Festuca*), the nucleotide sequence encoding it, the promoter from its gene and various uses of them. 4CL from fescue is considered to be the second "special technical feature".

3. Claims 1, 2, 6 to 12, 16, 17, 20a, 19b and 23 to 26 (partial) and claims 4, 14 and 22 (complete) are to cinnamoyl-CoA reductase (CCR) from ryegrass, the nucleotide sequence encoding it, the promoter from its gene and various uses of them. CCR from ryegrass is considered to be the third "special technical feature".

4. Claims 1, 6 to 11, 16, 17, 19b, 20a and 23 to 26 (partial) are to CCR from fescue, the nucleotide sequence encoding it, the promoter from its gene and various uses of them. CCR from fescue is considered to be the fourth "special technical feature".

5. Claims 1, 2, 6 to 12, 16, 17, 19b and 23 to 26 (partial) and claims 5 and 15 (complete) are to cinnamyl alcohol dehydrogenase (CAD) from ryegrass, the nucleotide sequence encoding it, the promoter from its gene and various uses of them. CAD from ryegrass is considered to be the fifth "special technical feature".

6. Claims 1, 6 to 11, 16, 17, 19b, and 23 to 26 (partial) are to CAD from fescue, the nucleotide sequence encoding it, the promoter from its gene and various uses of them. CAD from fescue is considered to be the sixth "special technical feature".

7. Claims 17, 18, 19b and 23 to 26 (partial) and claim 20b (complete) are to caffeic acid O-methyltransferase (OMT) gene promoter from ryegrass and various uses of it. Caffeic acid OMT from ryegrass is considered to be the seventh "special technical feature".

8. Claims 17, 18, 19b and 23 to 26 (partial) are to caffeic acid O-methyltransferase (OMT) gene promoter from fescue and various uses of it. Caffeic acid OMT from fescue is considered to be the eighth "special technical feature".

In order for there to be unity between the four types of enzymes claimed, they have to share a significant structural element, that is a structural element that defines the specific biological activity of the enzymes, and the significant structural element must be disclosed in the specification. No significant structural element has been identified as being shared by the four types of enzymes, hence there is lack of unity between the enzymes. In addition, all four types of enzymes are known in the prior art, for example, in US 5 952 486. Hence unity of invention is also lacking between the enzymes from ryegrass and the enzymes from fescue.

Note that the application as filed contains two claims numbered 19 and two claims numbered 20. To distinguish them, 19a and 20a refer to the first claims 19 and 20, and 19b and 20b refer to the second claims 19 and 20.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU01/00699

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	5 952 486	AU	44036/97	BR	9711745	EP	929 682
		US	5 850 020	WO	98/11205	ZA	9710451
		ZA	9810574	US	6 204 434		
WO	99/31243	US	6 252 135	ZA	9811568		
WO	98/39454	AU	63041/98	EP	970 222		
WO	93/05159	AU	16581/92	BR	9205934	CA	2 109 222
		EP	584 117	US	5 451 514	US	6 066 780
WO	93/24638	AU	43345/93	BR	9306445	EP	643 774
		US	5 633 439				

END OF ANNEX